INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/14866

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/07 A61K31/05 A61K31/327 A61K31/60 A61K31/203
A61K31/191 A61K33/04 A61P17/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 016, no. 470 (C-0990), 30 September 1992 (1992-09-30) & JP 04 169511 A (POLA CHEM IND INC;OTHERS: 01), 17 June 1992 (1992-06-17) abstract	1-14
X	WO 98/53822 A (THORNFELDT CARL R ;CELLERGY PHARMACEUTICALS INC (US)) 3 December 1998 (1998-12-03) claims 1-12; example 2	1-14

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2004	07/04/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Herrera, S

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/14866

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 13 and 14 (partly) are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/14866

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 04169511 A	17-06-1992	JP 2875374 B2	31-03-1999
WO 9853822 A	03-12-1998	US 6071543 A	06-06-2000
		AU 7717998 A	30-12-1998
		WO 9853822 A1	03-12-1998
		US 6482839 B1	19-11-2002

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 03 25 2390

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	PATENT ABSTRACTS OF JAPAN vol. 016, no. 470 (C-0990), 30 September 1992 (1992-09-30) & JP 04 169511 A (POLA CHEM IND INC;OTHERS: 01), 17 June 1992 (1992-06-17) * abstract *	1-14,16	A61K31/07 A61K31/05 A61K31/327 A61K31/60 A61K31/203 A61K31/191 A61K33/04 A61P17/10
X	WO 98 53822 A (THORNFELDT CARL R ;CELLERGY PHARMACEUTICALS INC (US)) 3 December 1998 (1998-12-03) * claims 1-12; example 2 *	1-16	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A61K
A61P

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	10 September 2003	Herrera, S

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

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INCOMPLETE SEARCH SHEET C

Application Number
EP 03 25 2390

Although claims 15 and 16 (partly) are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) searched completely:
1-14

Claim(s) searched incompletely:
15-16

Reason for the limitation of the search (non-patentable invention(s)):

Article 52 (4) EPC - Method for treatment of the human or animal body by therapy

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 03 25 2390

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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10-09-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 04169511 A	17-06-1992	JP 2875374 B2	31-03-1999
WO 9853822 A	03-12-1998	US 6071543 A	06-06-2000
		AU 7717998 A	30-12-1998
		WO 9853822 A1	03-12-1998
		US 6482839 B1	19-11-2002

EUROPEAN PATENT OFFICE

Patent Abstracts of Japan

PUBLICATION NUMBER : 04169511
PUBLICATION DATE : 17-06-92

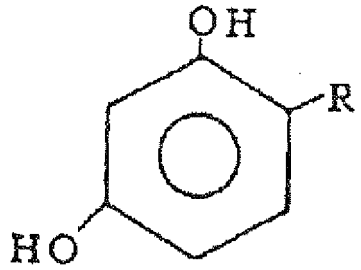
APPLICATION DATE : 31-10-90
APPLICATION NUMBER : 02291881

APPLICANT : KURARAY CO LTD;

INVENTOR : TAMAI HIRONOBU;

INT.CL. : A61K 7/00

TITLE : COSMETIC FOR COMMON ACNE



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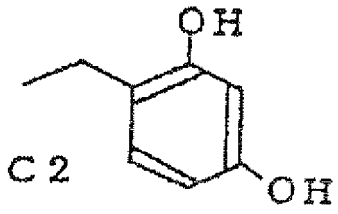

II

ABSTRACT : PURPOSE: To obtain a cosmetic for common acne, capable of exhibiting remarkably inhibitory effects on the common acne and excellent also in safety by blending a specific amount of a specified resorcinol derivative having antimicrobial action in a cosmetic.

CONSTITUTION: A cosmetic for common acne is obtained by blending a resorcinol derivative (e.g. resorcinol expressed by formula II) expressed by formula I (R is 2-12C straight-chain or branched alkyl) in an amount of at least 0.001wt.% (0.1-2wt.% is especially preferred) based on the total amount of the cosmetic composition for the common acne. The aforementioned cosmetic has powerful action on germs producing or worsening the common acne. The above- mentioned cosmetic for the common acne is used as cream, milky lotion, toilet water, pack, soap, etc.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/44 | A1

(11) International Publication Number: WO 98/53822

(43) International Publication Date: 3 December 1998 (03.12.98)

(21) International Application Number: PCT/US98/11270

(22) International Filing Date: 2 June 1998 (02.06.98)

(30) Priority Data:

60/047,360	2 June 1997 (02.06.97)	US
60/056,282	3 September 1997 (03.09.97)	US
09/089,302	1 June 1998 (01.06.98)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	09/089,302 (CIP)
Filed on	1 June 1998 (01.06.98)
US	60/047,360 (CIP)
Filed on	2 June 1997 (02.06.97)
US	60/056,282 (CIP)
Filed on	3 September 1997 (03.09.97)

(71) Applicant *(for all designated States except US)*: CELLERGY PHARMACEUTICALS INC. [US/US]; Suite 418, 1065 E. Hillsdale Boulevard, Foster City, CA 94404 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: THORNFELDT, Carl, R. [US/US]; 221 Crestview Drive, Nampa, ID 83686 (US).

(74) Agents: SMITH, Timothy, L. et al.; Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111-3834 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: PYRIDINE–THIOLS REVERSE MUCOCUTANEOUS AGING

(57) Abstract

This invention provides compositions and methods for preventing and reversing the signs and symptoms of intrinsic and photo aging. The compositions include one or more pyridine–thiols and tautomers with attached metallic moieties. Administration of the compounds to aging skin and mucous membranes in topical formulations, either as the only active ingredient or in combination with other known active ingredients, prevents and reverses aging symptoms. Additional compositions for preventing and reversing aging contain one or more sulfides and oxides of these same metallic ions, either alone or in combination with other molecules known or suspected to exhibit age reversing properties. Topical formulations containing both a pyridine–thiol and tautomers with attached metallic moiety and a metallic sulfide and/or metallic oxide effectively prevent and reverse the signs and symptoms of mucocutaneous aging.

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PYRIDINE-THIOLS REVERSE MUCOCUTANEOUS AGING

BACKGROUND OF THE INVENTION

Field of the Invention

This invention pertains to the field of treating and preventing signs and symptoms of aging.

Background

Therapeutic products comprising metallic moieties have been used for many years for a variety of skin diseases. These medications have continued to be used to treat one or several skin diseases. For example, zinc pyrithione (zinc pyridine-2-thiol-1-oxide) is a therapeutic molecule that is used as the active ingredient in the most widely distributed commercially available medicated shampoos for treatment of dandruff and seborrheic dermatitis. In the past year, this compound has been introduced by two companies in a topical leave-on product to treat scalp psoriasis. Zinc pyrithione has multiple mechanisms of action including antiproliferative, keratolytic, astringent, antibacterial and anti-yeast properties. Zinc undecylenate has also been used as an antifungal agent. Zinc oxide has also had a long history as a sunblock and skin protectant especially for the diaper area. Zinc lactate 0.15% is one component of a prescription product which also comprises erythromicin 2% in a topical therapy for acne vulgaris.

U.S. patent no. 4,307,089 discusses a formulation that contains zinc pyrithione and/or its tautomeric form combined with undecylenic acid and the use of the formulation to treat dandruff. U.S. patent no. 5,284,649 discusses the use of heavy metal

salts of hydroxypyridine thiones and their tautomeric forms, including zinc, zirconium, cadmium, tin, magnesium, sodium, calcium, aluminum and potassium pyrithione, as human deodorants.

Zinc is an essential mineral for animal cell growth and regeneration due to its integral structural role in certain enzymes especially proteases including carboxypeptidase A. Furthermore, the deoxyribonucleic acid (DNA) contains zinc finger binding domains utilized in transcription thus regulating gene activity. This element also functions as an enzyme activator, a coenzyme, and an antioxidant. Zinc and other bivalent ions including cobalt, copper, nickel, and manganese inhibit the binding of triiodothyronine to its nuclear receptor. Zinc, selenium, vanadium, and chromium all have documented insulin mimetic activity.

Selenium is a known antioxidant utilized as an immune modulator in naturpathic and lay medicine. Its major mechanism of action is via covalent binding to the key detoxification/antioxidant enzyme glutathione peroxidase. Multiple selenium sulfide shampoos have been on the prescription and over-the-counter markets for years to treat dandruff and seborrheic dermatitis. The difference between the two markets is that the prescription product has a much higher concentration of the selenium sulfide. These products are generally considered to be more effective than zinc pyrithione because of documented superior anti-microbial activity.

Multiple enzymes are known to require metallic ions as cofactors or are needed as catalysts. Several other of metals currently are or have been in the past used a human disease medicines. Arsenic was a major topical treatment for psoriasis prior to the advent of corticosteriods. Gallium formulations injected intravenously are used in human medical diagonstic tests. Copper and silver salts are the active ingredients in topical products for cleansing and deodorizing stomas and burns. Strontium has been reported to treat stinging/burning due to neurogenic inflammation but is associated with bone deposition and marrow suppression.

Use of these metallic compounds as therapeutic compounds would be expected to have serious drawbacks because several, including nickel, chromium, and cobalt, are potent contact sensitizers of the skin and mucous membranes. Iron is a potent oxidant inducing cell damage. Bromine often induces a characteristic dermatosis known as

bromoderma. High calcium levels in the stratum corneum inhibit normal barrier formation and desquamation.

Chronologically aged (intrinsic aging) mucocutaneous surfaces show a slight atrophy of the epidermis with straightening of the rete pegs thus weakening the dermal/epidermal junction measured by a decrease in the threshold for suction bullae. There is a moderate decrease in the number of Langerhans cells. Dryness of the skin is a common phenomenon. In the dermis there is lower cellularity and a decrease in elastic fibers and thus in skin elasticity. Capillaries are also fragile as evidenced by bruisability. Collagen metabolism is slower, and there is a progressive lowering in concentration of glycosaminoglycans. Wrinkling occurs, but it tends to be in the form of fine wrinkles that disappear temporarily with stretching. There is a decreased ability to mount inflammatory response and an increase in the time of healing after injury.

Photoaging induces deep wrinkles not erased by stretching, pigmentary alterations with areas of hyper- and hypopigmentation (actinic lentigines and leukodermas), and a variety of benign, premalignant, and malignant neoplasms. The dermis shows evidence of chronic inflammation with increased cellularity and enlarged fibroblasts. Elastotic degeneration occurs in which parts of the upper dermis is occupied by a basophilic fibrous material separating the dermis from the epidermis. This "grenz" zone is interpreted as a repair area. Glycosaminoglycan concentrations is increased, while elastin concentration is increased and arranged in atypical clumps. Collagen fibers are fragmented.

A need exists for methods and compositions that are effective in preventing and/or reversing signs and symptoms of aging. The present invention fulfills these and other needs.

SUMMARY OF THE INVENTION

The invention provides methods of treating or preventing symptoms and signs of aging on a mucocutaneous tissue. In some embodiments, the methods involve topically applying to an affected area of the mucocutaneous tissue a therapeutically effective amount of a topical formulation that contains a metal ion associated with a compound selected from the group consisting of a pyridine-thiol and a tautomer of a pyridine-thiol.

In additional embodiments, the invention provides methods for treating or preventing symptoms of aging on a mucocutaneous tissue by topically applying to an

affected area of the mucocutaneous tissue a therapeutically effective amount of a topical formulation comprising at least one of a metal oxide or a metal sulfide.

The invention also provides compositions that can be used to treat or prevent signs and symptoms of aging. In some embodiments, the topical formulations contain (a) a metal ion associated with a compound selected from the group consisting of a pyridine-thiol and a tautomer of a pyridine-thiol; and (b) one or more compounds which are effective in treating symptoms of aging of mucocutaneous tissue.

In other embodiments, the topical formulations contain (a) a metal cation and an anion selected from the group consisting of an oxide and a sulfide; and (b) one or more compounds which are effective in treating symptoms of aging of mucocutaneous tissue.

The invention also provides methods for treating or preventing signs or symptoms of aging by topically applying to an affected area of the mucocutaneous tissue a therapeutically effective amount of a topical formulation containing: (a) a metal ion associated with a compound selected from the group consisting of a pyridine-thiol and a tautomer of a pyridine-thiol; and (b) a metal oxide or a metal sulfide. Topical formulations that contain these ingredients are also provided.

DETAILED DESCRIPTION

Definitions

The term "therapeutically effective amount" or "effective amount" is used herein to denote any amount of a topical formulation which will cause a substantial improvement in a disease condition (such as a subsidence of a lesion, for example) when applied to the affected area. A single application can be sufficient, or the formulation can be applied repeatedly over a period of time. The amount will vary with the condition being treated, the stage of advancement of the condition, and the type and concentration of formulation applied. Appropriate amounts in any given instance will be readily apparent to those skilled in the art or capable of determination by routine experimentation.

A "cosmeceutical" is a product, typically non-prescription, that is utilized in the cosmetic industry which produces measurable structural changes in the skin and mucous membranes.

Description of the Preferred Embodiments

The present invention provides methods and compositions that are useful for treating or preventing signs and symptoms of aging. The compositions of the invention contain one or more anti-aging compounds that are metal ions complexed with either pyridine thiols or are metal sulfides or metal oxides. The compositions are typically applied to skin or mucous membranes to prevent or treat the aging symptoms, which can be a result of chronologic (intrinsic) aging or photoaging.

A. Anti-Aging Compounds and Formulations

The anti-aging compounds of the invention include pyridine-thiols, as well as tautomers of the pyridine thiols, that are associated with a metal ion. In other embodiments, the anti-aging compounds of the invention are metal ions associated with a sulfide ion or an oxide ion. In other embodiments, the formulations of the invention include combinations of the pyridine-thiol oxides and sulfides and tautomers thereof. The zinc pyrithione and selenium pyrithione combination are preferred. Furthermore, this invention includes metallic sulfides and metallic oxides in combination as well as with pyridine-thiol with attached metallic ion or its tautomers. Selenium sulfide with zinc pyrithione is preferred.

The metal ions that can be included in the anti-aging compounds of the invention are, for example, copper, manganese, vanadium, strontium, sodium, silver, cadmium, calcium, titanium, tin, gallium, germanium, scandium, arsenic, aluminum, magnesium, bromine, cobalt, nickel, chromium, potassium, and iron. Zirconium, zinc, strontium, silver, selenium, copper, manganese, gallium, titanium sodium, potassium, vanadium, magnesium, calcium, and arsenic are preferred. Zinc, strontium, silver, selenium and copper are most preferred.

1. Pyridine Thiols

In some embodiments, the formulations of the invention include pyridine-thiols and/or tautomers of the pyridine thiols. Examples of suitable pyridine thiols include, but are not limited to, zinc pyrithione, selenium pyrithione, silver pyrithione, and copper pyrithione. Zinc pyridine-2-thiol-1-oxide (pyrithione) is a preferred pyridine thiol. These organometallic compounds typically exist as bis adducts. For example, in a preferred embodiment, the empirical formula is $C_{10}H_8N_2OS_2Zn$. The synthesis of bis(2-pyridylthio)zinc 1,1' dioxide (*i.e.*, zinc pyridine-2-thiol-1-oxide) is outlined in British Patent

No. 761,171 and U.S. Patent Nos. 3,236,733 and 3,281,366 all of which are incorporated herein by reference.

2. Metal Sulfides and Oxides

The sulfides and oxides of the metallic ions that have activity against symptoms and signs of aging include, for example, any combination of a sulfide or an oxide moiety associated with a metal as set forth above. Particularly preferred compounds include selenium sulfide and zinc oxide.

3. Formulations and Dosages

Typically, the anti-aging compositions described herein will be in the form of a topical formulation for delivering the active ingredient. The formulation will typically contain the anti-aging compound in concentrations that range from about 0.001% to about 60.0% by weight, with about 0.025% to about 20.0% by weight preferred, and about 0.1% to about 5.0% by weight the most preferred. The formulations generally also include a non-toxic, pharmaceutically and/or cosmeceutically acceptable carrier. *See, e.g.*, DRUG : FACTS AND COMPARISONS, Published by Facts and Comparisons, A Wolters Kluwer Company (1997) and DERMATOLOGICAL FORMULATIONS: PERCUTANEOUS ABSORPTION, Barry (ed.), Marcel Dekker Inc. (1983).

The local absorption and efficacy of the anti-aging compounds can be further enhanced by incorporating an appropriate amount of an excipient which can allow increased penetration of, or assist in the delivery of therapeutic molecules across, the stratum corneum permeability barrier of the skin. Many of these penetration enhancing molecules are known to those trained in the art of topical formulation. Examples include humectants such as urea and glycols such as propylene glycol, alcohols including ethanol, fatty acids such as oleic acid, surfactants such as isopropyl myristate and sodium lauryl sulfate, pyrrolidones, glycerol monolaurate, sulfoxides, terpenes including menthol, amines, amides, alkanes, alkanols, Orgelase and water. Vegetable oils or botanical oils containing high unsaturated fatty acids, *e.g.* safflower oil, olive oil, avocado oil, wheat germ oil, *etc.* or other chemicals can also facilitate absorption and delivery of compounds.

Pharmaceutically and cosmeceutically acceptable carriers will include water, saline, buffers, and other compounds described, *e.g.*, in the MERCK INDEX, Merck & Co., Rahway, NJ. *See, also,* BIOREVERSIBLE CARRIERS IN DRUG DESIGN, THEORY AND

APPLICATION, Roche (ed.), Pergamon Press, (1987). Various considerations are described, *e.g.*, in Gilman *et al.* (eds) (1990) GOODMAN AND GILMAN'S: THE PHARMACOLOGICAL BASES OF THERAPEUTICS, 8th Ed., Pergamon Press; NOVEL DRUG DELIVERY SYSTEMS, 2nd Ed., Norris (ed.) Marcel Dekker Inc. (1989), and REMINGTON'S PHARMACEUTICAL SCIENCES, the full disclosures of which are incorporated herein by reference. For standard dosages of conventional pharmacological agents, see, *e.g.*, PHYSICIANS DESK REFERENCE (1997 Edition); and American Medical Association (1997) *Drug Evaluations* (Subscriptions).

The anti-aging compounds of the invention can be administered in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspensions, liposomes, nasal/aerosolized dosage forms, implants, injectable and infusible solutions. These agents can also be incorporated into various cosmetic and toiletry formulations (*See, e.g.,* Flick E.W. COSMETIC AND TOILETRY FORMULATIONS, 2nd Ed., Noyes Publications, 1989). The preferred form depends on the intended mode of administration and therapeutic or cosmetic application.

Dosage forms for the topical administration of the compositions of the invention include various mixtures and combinations that can be applied topically and will permit even spreading and absorption into the cutaneous and mucosal surfaces. Examples include sprays, mists, aerosols, lotions, creams, solutions, gels, ointments, pastes, unguents, emulsions and suspensions. The active compound can be mixed under sterile conditions with a cosmeceutically or pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required. Topical preparations can be prepared by combining the anti-aging compounds with conventional pharmaceutical and/or cosmeceutical diluents and carriers commonly used in topical dry, liquid, cream and aerosol formulations. Ointment and creams can, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Such bases can include water and/or an oil such as liquid paraffin or a vegetable oil such as peanut oil or castor oil. Thickening agents which can be used according to the nature of the base include soft paraffin, aluminum stearate, cetostearyl alcohol, propylene glycol, polyethylene glycols, woolfat, hydrogenated lanolin, beeswax, and the like. Lotions can be formulated with an aqueous or oily base and will, in general, also include one or more of the following: stabilizing agents, emulsifying agents, dispersing agents, suspending agents, thickening

agents, coloring agents, perfumes, and the like. Powders can be formed with the aid of any suitable powder base, *e.g.*, talc, lactose, starch, and the like. Drops can be formulated with an aqueous base or non-aqueous base, and can also include one or more dispersing agents, suspending agents, solubilizing agents, and the like.

The ointments, pastes, creams and gels also can contain excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays also can contain excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Solutions of anti-aging compound can be converted into aerosols or sprays by any of the known means routinely used for making aerosol inhalant pharmaceuticals. In general, such methods comprise pressurizing or providing a means of pressurizing a container of the solution, usually with an inert carrier gas, and passing the pressurized gas through a small orifice. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Multiple inactive ingredients are generally incorporated in topical formulations to improve cosmetic acceptability, and are optional ingredients in the formulations of this invention. Examples of ingredients are emulsifiers, humectants, surfactants, preservatives, fragrances, coloring agents, emollients, and fillers.

The topical pharmaceutical compositions can also include one or more preservatives or bacteriostatic agents, *e.g.*, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chlorides, and the like. The topical pharmaceutical compositions also can contain other active ingredients such as antimicrobial agents, particularly antibiotics, anesthetics, analgesics, and antipruritic agents.

One example of a topical formulation contains, in addition to the anti-aging agent, light mineral oil, sorbitol solution, hydroxyoctacosanyl hydroxystearate, methoxy PEG-22/dodecyl glycol copolymer, stearoxytrimethylsilane and stearic alcohol, dimethicone 50 cs, fragrance, methylparaben, edetate disodium, quarterium-15, butylates hydroxytoluene, citric acid (monohydrate) and purified water.

The dosage of a specific anti-aging compound depends upon many factors that are well known to those skilled in the art, for example, the particular compound; the condition being treated; the age, weight, and clinical condition of the recipient patient; and the experience and judgment of the clinician or practitioner administering the therapy. An effective amount of the compound is that which provides either subjective relief of symptoms or an objectively identifiable improvement as noted by the clinician or other qualified observer. The dosing range varies with the compound used, the route of administration and the potency of the particular compound.

Because the anti-aging compounds of the invention are each effective alone, the compositions can be essentially free of other agents that are effective against aging symptoms on mucocutaneous membranes. In some embodiments, however, the compositions include additional agents that are known, reported, or suspected to display anti-aging activity. Such molecules include, for example, keratolytics such as hydroxy acids and their lactones, ketoacids, phenolics, amino acids, carboxylic acids, antioxidants, vitamins A, C, E, certain nutrients, metallic elements, anti-inflammatory agents, and the esters, amides, aldehydes, salts, analogs, isomers and derivatives thereof. Examples of specific anti-aging active ingredients that can be additionally incorporated into formulations of this invention include, for example, alpha, beta, gamma and poly-hydroxy and keto acids as well as tretinoin, retinol, retinaldehyde, ascorbic acid, tocopherol, dicarboxylic acids, lactones of hydroxy acids, kojic acids, other carboxylic acids, including linoleic, compounds with a phenol ring as the primary active structure, derivatives of phenol, chloroacetic acids, corticosteroids, nonsteroidal anti-inflammatory agents, sulfones, catechins and other antioxidants, amino acids and other minerals, and the esters, amides, salts, analogs, aldehydes, isomers, and derivatives thereof.

In preferred embodiments, the additional anti-aging agents included in combination formulations of this invention include esters, ethers and amides of salicylic, benzilic, malic, citric, tartaric, pyruvic, glycolic, lactic, glucuronic, tropic, linoleic, linolenic, azelaic, kojic, ascorbic, mandelic, benzoic, acetic, formic, fumaric, oxalic, propanoic, succinic, galatonic, galacturonic, glucuronic,glyceric, mucic, succharic, tartaronic, allolactic, phenyllactic, tetrahydroxypentanoic and hexahydroxyheptanoic acids, gluconolactone, tocopherol, retinol, tretinoin, retinaldehyde, vitamin D analogs, glucocorticosteroids,

colchicine, trichlorocetic and dichloracetic acids, ibuprofen, ketoprofen, ketorolac, piroxicam, indomethacin, serine, alanine, glycine, arginine, phenol, thymol, menthol, eucalyptol, methylresorcinol, hexylresorcinol, resorcinol, 3-hydroxy butyric acid, 4-hydroxyvaleric acid, dapsone and epigallocatechingallate. The additional items in the preceding list are examples only; the list is not intended to be inclusive of all compounds that are known, reported, or suspected to display activity in reversing the signs of aging of the skin and mucous membranes.

The compounds of the invention which are effective in treating and/or preventing signs and symptoms of aging can also be used in conjunction with anti-inflammatory and other therapeutic agents. Examples of anti-inflammatory agents include, for example, concentrated inflammation modifiers as described in commonly assigned US Patent Application Ser. No. _____, filed on May 29, 1998 as Attorney Docket No. 010692-002510US.

B. Methods for Treating or Preventing Signs and Symptoms of Aging

The invention also provides methods for treating signs and symptoms of aging of the skin and mucosal membranes. The treatments involve administering an effective amount of an anti-aging compound of the invention as described herein, typically as a topical formulation. The formulations of this invention are generally applied to the locally affected diseased or abnormal skin or mucous membranes.

The methods described herein find use in the treatment and/or prevention of a variety of signs and symptoms of aging. Such signs and symptoms against which the methods are effective include, but are not limited to, wrinkling, irregular pigmentation, laxity, inelasticity, fragility, roughness, poor wound healing, and neoplasia.

To treat or prevent an aging-related condition of the skin or mucosal membrane, a composition that contains one or more of the compounds described herein is administered to the skin or mucosal membrane in an amount effective to modulate the condition. An effective amount can be determined by applying the compositions containing the compounds of the invention to test animal models.

EXAMPLES

The following examples are offered to illustrate, but not to limit the present invention.

EXAMPLE 1
Formulation A

A 0.25% zinc pyrithione lotion was produced by dissolving 2.5 mg of zinc-1-hydroxypyridine-2-thione (Sigma: St. Louis, MO) in 100 milliliters of 60% ethanol, 25% propylene glycol and 15% water. This emulsion was designed to be thoroughly shaken prior to topical application to affected mucocutaneous surface. Once applied, Formulation A was allowed to dry for 3 to 5 minutes; glycerin was then applied sparingly to cover the whole surface.

EXAMPLE 2
Application

Three middle aged patients afflicted with mild acne vulgaris with about 10 inflammatory lesions on each side of the face and moderate fine wrinkling, irregular pigmentation, and loss of elasticity were treated with Formulation A twice daily for 12 weeks. All patients experienced complete clearing of the acne lesions and noticeable decrease in the degree and number of wrinkles and pigmentation with improvement in elasticity.

EXAMPLE 3
Formulation B

Formulation A was adjusted to Formulation B by adding 5 mg of salicylic acid (Sigma: St. Louis, MO) by weight to make a 0.5% solution. Each application was performed as in Example 1 above.

EXAMPLE 4
Application

Two middle aged patients were treated with Formulation B twice daily for 16 weeks. Both experienced a moderate diminution of fine wrinkling, irregular pigmentation, and improved skin texture.

EXAMPLE 5
Formulation C

Formulation C was prepared by dissolving 25 milligrams of selenium sulfide (Sigma: St. Louis, MO) in 100 milliliters of 60% ethanol, 25% propylene glycol, and 15% water to make a 2.5% by weight selenium sulfide solution. Each application was performed as in Example 1 above.

EXAMPLE 6
Application

Two middle aged males suffered from skin aging experienced moderate improvement in all signs with twice daily application of Formulation C for 16 weeks.

EXAMPLE 7
Application

Three patients suffering from frequently recurrent facial seborrheic dermatitis and moderate signs of aging applied Formulation C twice daily for 16 weeks. There was complete clearing of the dermatitis with no recurrences during this period. All patients experienced moderately improved texture and diminished fine wrinkles.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

WHAT IS CLAIMED IS:

1. A method for treating a symptom of aging on a mucocutaneous tissue, the method comprising topically applying to an affected area of the mucocutaneous tissue a therapeutically effective amount of a topical formulation comprising a metal ion associated with a compound selected from the group consisting of a pyridine-thiol and a tautomer of a pyridine-thiol.

2. The method of claim 1, wherein the aging is selected from the group consisting of chronologic aging and photoaging.

3. The method of claim 1, wherein the metal ion is selected from the group consisting of zirconium, zinc, vanadium, titanium, tin, strontium, silver, sodium, selenium, scandium, potassium, magnesium, manganese, nickel, germanium, gallium, copper, calcium, cadmium, cobalt, chromium, iron, bromine, aluminum and arsenic.

4. The method of claim 1, wherein the topical formulation comprises about 0.001% to about 60% by weight pyridine-thiol and pyridine-thiol tautomer.

5. The method of claim 1, wherein the symptom of aging is selected from the group consisting of wrinkling, irregular pigmentation, laxity, inelasticity, fragility, roughness, poor wound healing, and neoplasia.

6. The method of claim 1, wherein the topical formulation comprises about 0.1% to about 5% by weight of a compound selected from the group consisting of zinc pyrithione, silver pyrithione, selenium pyrithione, and copper pyrithione.

7. The method of claim 6, wherein the topical formulation comprises about 2.5% by weight of zinc pyrithione.

8. The method of claim 1, wherein the topical formulation is applied in a form selected from the group consisting of a spray, a mist, an aerosol, a solution, a lotion, a gel, a cream, an ointment, a paste, an unguent, an emulsion, and a suspension.

9. The method of claim 1, wherein the topical formulation further comprises one or more additional compounds which are effective in treating symptoms of aging.

10. The method of claim 9, wherein the additional compound is selected from the group consisting of alpha-, beta-, gamma-, and polyhydroxy and keto acids, retinol, retinaldehyde, tretinoin, ascorbic acid, tocopherol, dicarboxylic acids, kojic acids, other carboxylic acids, chloroacetic acids, compounds having a phenol ring as an primary active structure, derivatives of phenol, corticosteroids, nonsteroidal anti-inflammatory agents, sulfones, catechins and other antioxidants, amino acids, other minerals and nutrients, lactones, and esters, amides, salts, analogs, aldehydes, isomers and derivatives thereof.

11. The method of claim 10, wherein the additional compound is present in the topical formulation at a concentration of 0.01% to 99.9% by weight.

12. The method of claim 9, wherein the additional compound is selected from the group consisting of salicylic, benzilic, malic, citric, tartaric, tropic, glucuronic, mandelic, benzoic, acetic, formic, fumaric, oxalic, propanoic, succinic, galactonic, galacturonic, glucoronic, glyceric, mucic, succharic, tartaronic, allolactic, phenyllactic, pyruvic, glycolic, lactic, linoleic, linolenic, azelaic, kojic, ascorbic, trichloroacetic, and dichloracetic, tetrahydroxypentanoic and hexahydroxyheptanoic acids, glucoconolactone, tocopherol, retinol, retinaldehyde, tretinoin, vitamin D analogs, trichlorocetic acid, glucocorticosteroids, colchicine, ibuprofen, ketoprofen, ketorolac, piroxicam, indomethacin, serine, alanine, glycine, phenol, arginine, thymol, dapsone, menthol, eucalyptol, resorcinol, methyl resorcinol, hexyl resorcinol, 3-hydroxy butyric acid, 4-hydroxyvaleric acid, epigallocatechingallate, and esters, ethers, amides, analogs, derivatives, aldehydes, isomers and salts thereof.

13. The method of claim 12, wherein the additional compound is present in the topical formulation at a concentration of 0.5% to 30.0% by weight.

14. A topical formulation for treating a symptom of aging on a mucocutaneous tissue, the formulation comprising:

(a) a metal ion associated with a compound selected from the group consisting of a pyridine-thiol and a tautomer of a pyridine-thiol; and

(b) one or more compounds which are effective in treating symptoms of aging of mucocutaneous tissue.

15. The topical formulation of claim 14, wherein the metal ion is selected from the group consisting of zirconium, zinc, vanadium, titanium, tin, strontium, silver, sodium, selenium, scandium, potassium, magnesium, manganese, nickel, germanium, gallium, copper, calcium, cadmium, cobalt, chromium, iron, bromine, aluminum and arsenic.

16. The topical formulation of claim 14, wherein the topical formulation comprises about 0.1% to about 5% by weight of a compound selected from the group consisting of zinc pyrithione, silver pyrithione, selenium pyrithione, and copper pyrithione.

17. The topical formulation of claim 14, wherein the additional compound is selected from the group consisting of salicylic, benzilic, malic, citric, tartaric, tropic, glucuronic, mandelic, benzoic, acetic, formic, fumaric, oxalic, propanoic, succinic, galactonic, galacturonic, glucoronic, glyceric, mucic, succharic, tartaronic, allolactic, phenyllactic, pyruvic, glycolic, lactic, linoleic, linolenic, azelaic, kojic, ascorbic, trichloroacetic, and dichloracetic, tetrahydroxypentanoic and hexahydroxyheptanoic acids, glucoconolactone, tocopherol, retinol, retinaldehyde, tretinoin, vitamin D analogs, trichlorocetic acid, glucocorticosteroids, colchicine, ibuprofen, ketoprofen, ketorolac, piroxicam, indomethacin, serine, alanine, glycine, phenol, arginine, thymol, dapsone, menthol, eucalyptol, resorcinol, methyl resorcinol, hexyl resorcinol, 3-hydroxy butyric acid, 4-hydroxyvaleric acid, epigallocatechingallate, and esters, ethers, amides, analogs, derivatives, aldehydes, isomers and salts thereof.

18. A method for treating a symptom of aging on a mucocutaneous tissue, the method comprising topically applying to an affected area of the mucocutaneous tissue a

therapeutically effective amount of a topical formulation comprising at least one of a metal oxide or a metal sulfide.

19. The method of claim 18, wherein the aging is selected from the group consisting of chronologic aging and photoaging.

20. The method of claim 18, wherein the metal ion is selected from the group consisting of zirconium, zinc, vanadium, titanium, tin, strontium, silver, sodium, selenium, scandium, potassium, magnesium, manganese, nickel, germanium, gallium, copper, calcium, cadmium, cobalt, chromium, iron, bromine, aluminum and arsenic.

21. The method of claim 18, wherein the topical formulation comprises between about 0.001% and about 60% by weight of the metal oxide or metal sulfide.

22. The method of claim 21, wherein the topical formulation comprises from about 0.025% to about 20% by weight of the metal oxide or metal sulfide.

23. The method of claim 22, wherein the topical formulation comprises from about 0.1% to about 5% by weight of zinc oxide or selenium sulfide.

24. The method of claim 23, wherein the topical formulation comprises about 0.2% by weight of selenium sulfide.

25. The method of claim 18, wherein the symptom of aging is selected from the group consisting of wrinkling, irregular pigmentation, laxity, inelasticity, fragility, roughness, poor wound healing, and neoplasia.

26. The method of claim 18, wherein the topical formulation is applied in a form selected from the group consisting of a spray, a mist, an aerosol, a solution, a lotion, a gel, a cream, an ointment, a paste, an unguent, an emulsion, and a suspension.

27. The method of claim 18, wherein the topical formulation further comprises one or more additional compounds which are effective in treating symptoms of aging.

28. The method of claim 27, wherein the additional compound is selected from the group consisting of alpha-, beta-, gamma-, and polyhydroxy and keto acids, retinol, retinaldehyde, tretinoin, ascorbic acid, tocopherol, dicarboxylic acids, kojic acids, other carboxylic acids, chloroacetic acids, compounds having a phenol ring as an primary active structure, derivatives of phenol, corticosteroids, nonsteroidal anti-inflammatory agents, sulfones, catechins and other antioxidants, amino acids, other minerals and nutrients, lactones, and esters, amides, salts, analogs, aldehydes, isomers and derivatives thereof.

29. The method of claim 27, wherein the additional compound is selected from the group consisting of salicylic, benzilic, malic, citric, tartaric, tropic, glucuronic, mandelic, benzoic, acetic, formic, fumaric, oxalic, propanoic, succinic, galactonic, galacturonic, glucoronic, glyceric, mucic, succharic, tartaronic, allolactic, phenyllactic, pyruvic, glycolic, lactic, linoleic, linolenic, azelaic, kojic, ascorbic, trichloroacetic, and dichloracetic, tetrahydroxypentanoic and hexahydroxyheptanoic acids, glucoconolactone, tocopherol, retinol, retinaldehyde, tretinoin, vitamin D analogs, trichlorocetic acid, glucocorticosteroids, colchicine, ibuprofen, ketoprofen, ketorolac, piroxicam, indomethacin, serine, alanine, glycine, phenol, arginine, thymol, dapsone, menthol, eucalyptol, resorcinol, methyl resorcinol, hexyl resorcinol, 3-hydroxy butyric acid, 4-hydroxyvaleric acid, epigallocatechingallate, and esters, ethers, amides, analogs, derivatives, aldehydes, isomers and salts thereof.

30. A topical formulation comprising:

(a) a metal cation and an anion selected from the group consisting of an oxide and a sulfide; and

(b) one or more compounds which are effective in treating symptoms of aging of mucocutaneous tissue.

31. The topical formulation of claim 30, wherein the metal cation is selected from the group consisting of zirconium, zinc, vanadium, titanium, tin, strontium, silver, sodium, selenium, scandium, potassium, magnesium, manganese, nickel, germanium, gallium, copper, calcium, cadmium, cobalt, chromium, iron, bromine, aluminum and arsenic.

32. The topical formulation of claim 31, wherein the topical formulation comprises one or more compounds selected from the group consisting of zinc oxide and selenium sulfide.

33. The topical formulation of claim 30, wherein the additional compound is selected from the group consisting of alpha-, beta-, gamma-, and polyhydroxy and keto acids, retinol, retinaldehyde, tretinoin, ascorbic acid, tocopherol, dicarboxylic acids, kojic acids, other carboxylic acids, chloroacetic acids, compounds having a phenol ring as an primary active structure, derivatives of phenol, corticosteroids, nonsteroidal anti-inflammatory agents, sulfones, catechins and other antioxidants, amino acids, other minerals and nutrients, lactones, and esters, amides, salts, analogs, aldehydes, isomers and derivatives thereof.

34. A method for treating a symptom of aging on a mucocutaneous tissue, the method comprising topically applying to an affected area of the mucocutaneous tissue a therapeutically effective amount of a topical formulation comprising:

(a) a metal ion associated with a compound selected from the group consisting of a pyridine-thiol and a tautomer of a pyridine-thiol; and

(b) a metal oxide or a metal sulfide.

35. A topical formulation for treating a symptom of aging on a mucocutaneous tissue, the topical formulation comprising:

(a) a metal ion associated with a compound selected from the group consisting of a pyridine-thiol and a tautomer of a pyridine-thiol; and

(b) a metal oxide or a metal sulfide.

International application No.
PCT/US98/11270

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/44
US CL : 514/354

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/354

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and CAS ONLINE: pyridine-thiol or pyrithione and age, aging, wrink?, bruis?, dryn?, elast?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A --- Y	Chem. abstr., Vol. 106, 24 October 1986, (Columbus, OH, USA), the abstract No. 106:55622, SAKAMOTO, T. et al 'Shampoos Containing Vitamin E Acetate and Dandruff-Controlling Agents.' JP 61238716 A2.	1-13, 18-29, 34 --- 14-17, 30-33, 35

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SKIN CONDITIONING COMPOSITIONS CONTAINING COMPOUNDS FOR MIMICKING THE EFFECT ON SKIN OF RETINOIC ACID

(57) Abstract: A skin care product comprising from about 0.001 % to about 10 % of a retinoid, in combination with 0.0001 % to about 50 % of a combination of retinoid boosters.

WO 02/02074 A2

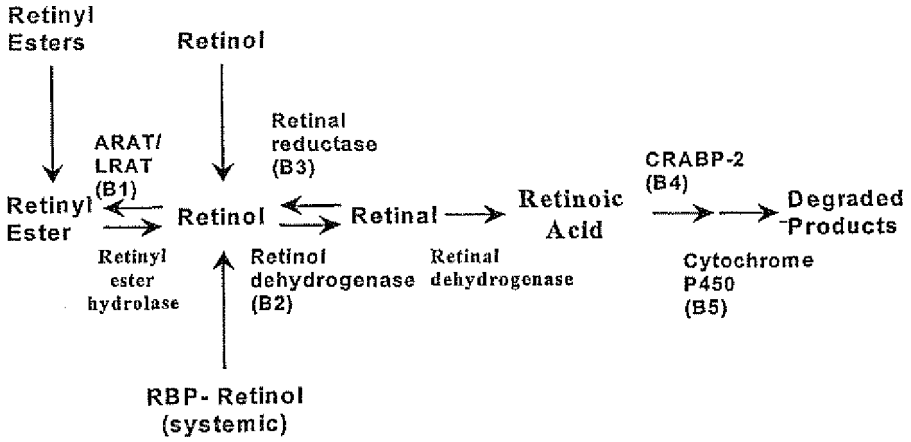
SKIN CONDITIONING COMPOSITIONS CONTAINING COMPOUNDS FOR MIMICKING THE EFFECT ON SKIN OF RETINOIC ACID

The present invention relates to cosmetic skin conditioning compositions containing certain compounds which mimic the effect on skin of retinoic acid.

Retinol (vitamin A) is an endogenous compound which occurs naturally in the human body, and is essential for normal epithelial cell differentiation. Natural and synthetic vitamin A derivatives have been used extensively in the treatment of a variety of skin disorders and have been used as skin repair or renewal agents. Retinoic acid has been employed to treat a variety of skin conditions, e.g., acne, wrinkles, psoriasis, age spots and discoloration. See e.g., Vahlquist, A. et al., *J. Invest. Dermatol.*, Vol. 94, Holland D.B. and Cunliffe, W.J. (1990), pp. 496-498; Ellis, C.N. et al., "Pharmacology of Retinols in Skin", Vasel, Karger, Vol. 3, (1989), pp. 249-252; Lowe, N.J. et al., "Pharmacology of Retinols in Skin", Vol. 3, (1989), pp. 240-248; PCT Patent Application No. WO 93/19743.

It is believed that retinol esters and retinol are enzymatically converted in the skin into retinoic acid according to the following mechanism:

Retinol metabolism in the epidermis: enzyme names



The present invention is based on the discovery that certain compounds enhance the conversion of retinyl esters and retinol to retinoic acid. The compounds are collectively termed "boosters" and are coded as groups B1 to B5 according to the boosting mechanism of the particular compound. The mechanism of the booster groups is as follows: inhibiting ARAT/LRAT (AcylCoenzymeA(CoA): retinol acyl transferase/Lecithin: retinol acyl transferase) activity (B1), enhancing retinol dehydrogenase activity (B2), inhibiting retinal reductase activity (B3), antagonising CRABP-II (Cellular Retinoic Acid Binding Protein II) binding of retinoic acid (B4) and inhibiting cytochrome P450 dependent retinoic acid oxidation (B5).

The boosters alone or in combination with each other potentiate the action of retinoids by increasing the conversion of the retinoids to retinoic acid and preventing

the degradation of retinoic acid. The boosters act in conjunction with a retinoid (e.g. retinol, retinyl esters, retinal, retinoic acid), the latter being present endogenously in the skin. The preferred compositions, however, include a retinoid in the composition, co-present with a booster or a combination of boosters, to optimise performance.

Several patents by Granger et al describe the use of retinoid boosters in cosmetic compositions to improve the efficacy of retinol and retinyl esters (US patent numbers: 5759556, 5756109, 5747051,5716627, 5811110, 5536740, 5747051, 5599548, 5955092, 5885595, 5759556, 5693330). The boosters described in these patents are restricted to class B1 and B5. Furthermore Johnson & Johnson have a series of patents which describe the use of molecules which fall into class 5 booster molecules (U.S. 5028628; U.S. 5037829; U.S. 5151421; U.S. 476852; U.S. 5500435; U.S. 5583136; U.S. 5612354).

The molecules which act as retinoid boosters are common ingredients in cosmetic products. There is considerable prior art describing their use in cosmetic compositions. There is substantial prior art describing the use of two or more of these molecules in the same composition. Some of the examples of the prior art are as in US 5,665,367, US 5747049, US 5853705, US 5766575, and US 5849310.

However, the prior art does not describe synergy arising from combinations of booster molecules. This observation of a synergistic boosting of retinoid activity from

combinations of booster molecules was an unexpected finding. The prior art does not describe optimal concentrations or ratios of booster molecules or ratios of booster molecules to that of retinoids. Thus, the present invention is novel in that by combining cosmetic retinoids with booster molecules, at the most appropriate concentrations or ratios, a substantial improvement in efficacy of the retinoids is obtained.

The classes of boosters suitable for use in the present invention include but are not limited to the boosters listed in Tables B1 through to B5.

Best Groups of Boosters

B1 Compounds

1. Fatty Acid Amides	These are readily commercially available and have the added advantage of being surfactants and thus help generate emulsions suitable for cosmetic preparations.
2. Ceramides	These can additionally act as precursors of stratum corneum barrier ceramides.
3. Carotenoids	These can offer some UV protection and act as natural colorants.
4. Flavanoids	Natural antioxidants.
5. Cyclic fragrances	These are readily commercially available and additionally can be used to fragrance the product.
6. Non-cyclic fragrances	These can be used to fragrance the product.
7. Phospholipid analogues	These can be utilised by skin cells to nourish the generation of barrier components.
8. Ureas	These are readily commercially available and can also act as preservatives for the product.

B2 Compounds

1. Phosphatidyl choline	Most preferred as most active activator of Retinol Dehydrogenase
2. Sphingomyelin	

B3 Compounds

Arachidonic Acid Linoleic Acid Linolenic Acid Myristic Acid	Fatty Acids which can be useful in maintaining stratum corneum barrier
Linoleic Acid Linolenic Acid	Essential Fatty Acids
Arachidonic Acid Myristic Acid	Non-essential fatty acids
Glycyrrhetinic Acid	Polycyclic triterpene carboxylic acid which is readily obtained from plant sources.
Phosphatidyl ethanolamine	Can be incorporated into cellular membranes.

B4 Compounds

Hexadecanedioic acid 12-hydroxystearic acid Isostearic acid	Saturated fatty acids.
Linseed oil Elaidic acid	Unsaturated fatty acids
Elaidic acid Isostearic acid Hexadecanedioic acid	Solid at room temperature
Linseed oil 12-hydroxystearic acid	Liquid at room temperature

B5 Compounds

Bifonazole Climbazole Clotrimazole Econazole Ketoconazole Miconazole	Antimicotics
Climbazole	Readily commercially available
Lauryl hydroxyethylimidazoline	Compounds which are readily commercially available and have the added advantage of being surfactants and thus help generate emulsions suitable for cosmetic preparations.
Quercetin	Naturally occuring flavanoid which has antioxidant properties.
Coumarin	Natural colorant
Quinolines Isoquinolines	
Metyrapone	

The present invention includes, in part, a skin conditioning composition containing from about 0.0001% to about 50%, preferably from 0.001% to 10%, most preferably from 0.001% to 5% by weight of the composition of a booster or combination of boosters and a cosmetically acceptable vehicle.

The boosters or combinations thereof included in the inventive compositions are selected from the group consisting of:

(a) a booster selected from the group consisting of B2; B3; B4;

(b) binary combinations of boosters selected from the group consisting of:

B1/B2; B1/B3; B1/B4; B1/B5; B2/B3, B2/B4; B2/B5, B3/B4; B3/B5; B4/B5

(c) ternary combinations of boosters selected from the group consisting of:
B1/B2/B3; B1/B2/B4; B1/B2/B5; B1/B3/B4; B1/B3/B5; B1/B4/B5; B2/B3/B4; B2/B3/B5; B2/B4/B5; B3/B4/B5

(d) quaternary combinations of boosters selected from the group consisting of:
B1/B2/B3/B4; B1/B2/B3/B5; B1/B2/B4/B5; B1/B3/B4/B5; B2/B3/B4/B5;

and

(e) a combination of five groups of boosters: B1/B2/B3/B4/B5.

The preferred compositions include from about 0.001% to about 10%, by weight of the composition of a retinoid.

The compounds included in the present invention as boosters are selected based on the ability of such compounds to pass, at a certain concentration listed in Table A, in-vitro Assays for a specific enzymes as described below under sections 2.1 through to 2.7. Such a booster is included in the present invention even if it is not explicitly mentioned herein. Put another way, if a compound inhibits or enhances sufficiently an enzyme in an assay described below, it will act in combination with a retinoid to mimic the effect on keratinocytes (skin cells) of retinoic acid, and thus it is included within the scope of the present invention.

The term "conditioning" as used herein means prevention and treatment of dry skin, acne, photo-damaged skin, appearance of wrinkles, age spots, aged skin, increasing stratum corneum flexibility, lightening skin colour, controlling sebum excretion and generally increasing the quality of skin. The composition may be used to improve skin desquamation and epidermal differentiation.

The presence of the selected compounds in the inventive product substantially improves the performance of a retinoid.

The inventive compositions contain, as a preferred ingredient, a retinoid, which is selected from retinyl esters, retinol, retinal and retinoic acid, preferably retinol or retinyl ester. The term "retinol" includes the following isomers of retinol: all-trans-retinol, 13-cis-retinol, 11-cis-retinol, 9-cis-retinol, 3,4-didehydro-retinol, 3,4-didehydro-13-cis-retinol; 3,4-didehydro-11-cis-retinol; 3,4-didehydro-9-cis-retinol. Preferred isomers are all-trans-retinol, 13-cis-retinol, 3,4-didehydro-retinol, 9-cis-retinol. Most preferred is all-trans-retinol, due to its wide commercial availability.

Retinyl ester is an ester of retinol. The term "retinol" has been defined above. Retinyl esters suitable for use in the present invention are C_1-C_{30} esters of retinol, preferably C_2-C_{20} esters, and most preferably C_2, C_3, and C_{16} esters because they are more commonly available. Examples of retinyl esters include but are not limited to: retinyl palmitate, retinyl

formate, retinyl acetate, retinyl propionate, retinyl butyrate, retinyl valerate, retinyl isovalerate, retinyl hexanoate, retinyl heptanoate, retinyl octanoate, retinyl nonanoate, retinyl decanoate, retinyl undecandate, retinyl laurate, retinyl tridecanoate, retinyl myristate, retinyl pentadecanoate, retinyl heptadeconoate, retinyl stearate, retinyl isostearate, retinyl nonadecanoate, retinyl arachidonate, retinyl behenate, retinyl linoleate, and retinyl oleate.

The preferred ester for use in the present invention is selected from retinyl palmitate, retinyl acetate and retinyl propionate, because these are the most commercially available and therefore the cheapest. Retinyl linoleate and retinyl oleate are also preferred due to their efficacy.

Retinol or retinyl ester is employed in the inventive composition in an amount of from about 0.001% to about 10%, preferably in an amount of from about 0.01% to about 1%, most preferably in an amount of from about 0.01% to about 0.5%.

The essential ingredient of the inventive compositions is a compound which passes in vitro Assays described below in sections 2.1 through to 2.7. A compound suitable for use in the present invention inhibits or enhances at a concentration listed in Table A an enzyme to at least a broad % listed in Table A.

Section A: Identification of Booster:

TABLE A
Booster Test Concentrations and % Inhibition/Increase

ARAT / LRAT Assay *(To identify B1 boosters)*

Invention	Compound Concentration	% Inhibition
Broad	100 µM	> 10%
Preferred	100 µM	> 25%
Most Preferred	100 µM	> 40%
Optimum	100 µM	> 50%

Retinol Dehydrogenase Assay *(To identify B2 boosters)*

Invention	Compound Concentration	% Increase
Broad	100 µM	> 10%
Preferred	100 µM	> 15%
Most Preferred	100 µM	> 20%
Optimum	100 µM	> 25%

Retinal Reductase Assay *(To identify B3 boosters)*

Invention	Compound Concentration	% Inhibition
Broad	100 µM	> 5%
Preferred	100 µM	> 10%
Most Preferred	100 µM	> 20%
Optimum	100 µM	> 35%

CRABPII Antagonist Assay *(To identify B4 boosters)*

Invention	Compound : Retinoic acid Ratio	% Inhibition
Broad	7000 : 1	> 25%
Preferred	7000 : 1	> 50%
Most Preferred	70 : 1	> 25%
Optimum	70 : 1	> 50%

Retinoic Acid Oxidation Assay *(To identify B5 boosters)*

Invention	Compound Concentration	% Inhibition
Broad	100 µM	> 25%
Preferred	100 µM	> 45%
Most Preferred	100 µM	> 70%
Optimum	100 µM	> 80%

The in vitro Microsomal Assays employed for determining the suitability of the inclusion of the compound in the inventive compositions are as follows:

1. Materials

All-trans-retinol, all-trans-retinoic acid, palmitoyl-CoA, dilauroyl phosphatidyl choline, NAD, and NADPH were purchased from Sigma Chemical Company. Stock solutions of retinoids for the microsomal assays were made up in HPLC grade acetonitrile. All retinoid standard stock solutions for HPLC analysis were prepared in ethanol, stored under atmosphere of N_2 at -70°C and maintained on ice under amber lighting when out of storage. Other chemicals and the inhibitors were commercially available from cosmetic material suppliers or chemical companies such as Aldrich or International Flavours and Fragrances.

2. Methods

2.1 Isolation of RPE microsomes (modified from (1))

50 frozen hemisected bovine eyecups, with the retina and aqueous humor removed were obtained from W. L. Lawson Co.,

Lincoln, NE, USA. The eyes were thawed overnight and the colored iridescent membrane was removed by peeling with forceps. Each eyecup was washed with 2x 0.5mL cold buffer (0.1M PO4 / 1mM DTT / 0.25M sucrose, pH 7) by rubbing the darkly pigmented cells with an artist's brush or a rubber policeman. The cell suspension was added to the iridescent membranes and the suspension was stirred for several minutes in a beaker with a Teflon stir bar. The suspension was filtered through a coarse filter (Spectra/Por 925µ pore size polyethylene mesh) to remove large particles, and the resulting darkly colored suspension was homogenized using a Glas-Col with a motor driven Teflon homogenizer.

The cell homogenate was centrifuged for 30 min. at 20,000g (Sorvaal model RC-5B centrifuge with an SS34 rotor in 2.5x10cm tubes at 14,000 RPM). The resulting supernatant was subjected to further centrifugation for 60 min. at 150,000g (Beckman model L80 Ultracentrifuge with an SW50.1 rotor in 13x51mm tubes at 40,000 RPM). The resulting pellets were dispersed into ~5mL 0.1M PO_4 / 5mM DTT, pH 7 buffer using a Heat Systems Ultrasonics, Inc. model W185D Sonifier Cell Disruptor, and the resulting microsomal dispersion was aliquoted into small tubes and stored at -70°C. The protein concentrations of the microsomes were determined using the BioRad Dye binding assay, using BSA as a standard.

2.2 Isolation of rat liver microsomes (4)

Approximately 6 grams of frozen rat liver (obtained from Harlan Sprague Dawley rats from Accurate Chemical and Scientific Corp.) was homogenized in 3 volumes of 0.1M tris /

0.1M KCl / 1mM EDTA / 0.25M sucrose, pH 7.4 buffer using a Brinkmann Polytron. The resulting tissue suspension was further homogenized in the motor driven Teflon homogenizer described above. The resulting homogenate was successively centrifuged for 30 min. at 10,000g, 30 min. at 20,000g, and 15 min. at 30,000g, and the resulting supernatant was ultra-centrifuged for 80 min. at 105,000g. The pellet was sonicated in ~5mL of 0.1M PO4 / 0.1mM EDTA / 5mM $MgCl_2$, pH 7.4 buffer as described above and stored as aliquots at -70°C. Protein concentrations were determined as described above.

2.3 Assay for ARAT and LRAT activity (To identify B1)

The procedure below was a modification of a method described in the literature (2). The following buffer was prepared and stored at 4°C: 0.1M PO_4 / 5mM dithiothreitol, pH 7.0 (PO_4/DTT). On the day of the assay, 2mg BSA per mL of buffer was added to give a PO_4 / DTT / BSA working buffer. 1mM retinol substrate was prepared in acetonitrile and stored in amber bottles under nitrogen gas at -20°C. Solutions of 4mM Palmitoyl-CoA in working buffer (stored in aliquots) and 4mM dilauroyl phosphatidyl choline in ethanol were prepared and stored at -20°C. Inhibitors were prepared as 10mM stock solutions in H_2O, ethanol, acetonitrile or DMSO. The quench solution was prepared using pure ethanol containing 50µg/mL butylated hydroxytoluene (BHT), and a hexane solution containing 50µg/mL BHT was used for the extractions.

To a 2 dram glass vial, the following were added in order: PO_4 / DTT / BSA buffer to give a total volume of 500µL, 5µL acyl donor (4mM palmitoyl-CoA and/or dilauroyl phosphatidyl

choline), 5µL inhibitor or solvent blank (10mM stock or further dilutions) followed by approximately 15µg of RPE microsomal protein (approximately 15µL of a ~1mg/mL microsomal protein aliquot). The mixture was incubated for 5 min. at 37°C to equilibrate the reaction temperature and then 5µL 1mM retinol was added. The vials were capped, vortexed for 5 seconds and incubated for 30-90 minutes at 37°C. The reaction was quenched by adding 0.5mL ethanol/BHT. The retinoids were extracted by adding 3mL hexane/BHT, vortexing the tubes for several seconds several times and centrifuging the tubes at low speed for 5 min. to quickly separate the layers. The upper hexane layer was removed into a clean vial, and the aqueous layer re-extracted with another 3mL hexane/BHT, as described above. The hexane layers were combined, and the hexane evaporated by drying at 37°C under a stream of nitrogen gas on a heated aluminum block. The dried residue was stored at -20°C until HPLC analysis. The amount of retinyl palmitate and retinyl laurate was quantitated for ARAT and LRAT activity, respectively, by integration of the HPLC signal as described below.

Note that the incubation solution contains 40µM acyl donor, 100µM or less inhibitor, 10µM retinol, approximately 30µg/mL microsomal protein, and nearly 0.1M PO_4/ pH 7 / 5mM DTT / 2mg/mL BSA. All steps subsequent to the addition of retinol were done in the dark or under amber lights.

2.4 Assay for Retinol Dehydrogenase Activity (To identify B2)

The following stock solutions were prepared:

50mM KH2PO4, pH 7.4 buffer, sterile filtered.
10mM all trans Retinol (Sigma R7632) in DMSO.
200mM Nicotinamide adenine dinucleotide phosphate, sodium salt (NADP) (Sigma N0505) in sterile water.
40mM test compound in appropriate solvent (water, buffer, ethanol, chloroform or DMSO).
1:10 dilution of rat liver Microsomes in 50mM KH2PO4, pH 7.4 buffer (4µg/µl).

In a two-dram glass vial with screw cap, the following were added in order:

Buffer to give a final volume of 400µl
25µl diluted Microsomes (final = 100µg) - boiled Microsomes were used for controls and regular Microsomes for test samples.
4µl of 200mM NADP (final = 2mM)
1µl of 40mM test compound (final = 100µM)
8µl of 10mM retinol (final = 200µM)

The vials were incubated in a 37°C shaking water bath for 45 minutes. 500µl ice-cold ethanol was added to each vial to quench the reaction. The retinoids were extracted twice with ice cold hexane (2.7ml per extraction). Retinyl acetate (5µl of a 900µM stock) was added to each vial during the first extraction as a means of monitoring the extraction efficiency in each sample. Samples were vortexed for ten seconds before gently centrifuging for five minutes at 1000rpm, 5°C in a Beckman GS-6R centrifuge. The top hexane layer containing the retinoids was removed from the aqueous layer after each extraction to a clean two-dram vial. The hexane was

evaporated off under a gentle stream of nitrogen gas. The dried residue was then stored at -20°C until HPLC analysis.

2.5 Assay for Retinal Reductase Activity (To identify B3)

All stock solution were prepared as above with the following substitutions:

10mM all trans Retinaldehyde (Sigma R2500) in DMSO - instead of retinol.
200mM, Nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt (NADPH) (Sigma N7505) in sterile water - instead of NADP.

In a two-dram glass vial with screw cap, add the following in order:

Buffer to give a final volume of 400µl
25µl diluted Microsomes (final = 100µg) - use boiled Microsomes for controls and regular Microsomes for test samples.
4µl of 200mM NADPH (final = 2mM)
1µl of 40mM test compound (final = 100µM)
3µl of 10mM retinaldehyde (final = 75µM)

Follow the same incubation and extraction procedure as detailed above.

2.6 Assay for CRABPII antagonists (To identify B4)

2.6.1. Synthesis of CRABPII

a. System of expression

The gene CRABPII was cloned in pET 29a-c(+) plasmid (Novagen). The cloned gene was under control of strong bacteriophage T7 transcription and translation signals. The source of T7 polymerase was provided by the host cell E.coli BLR(DE3)pLysS (Novagen). The latter has a chromosomal copy of T7 polymerase under lacUV5 control, induced by the presence of IPTG.

The plasmid was transferred into E. coli BLR(DE3)pLysS cells by transformation according to the manufacturer protocol (Novagen).

b. Induction

An overnight culture of the transformed cells was diluted 1:100 into 2xYT containing 50 µg/mL kanamycin and 25µg/mL chloramphenicol. The cells grew while shaking at 37°C until the OD at 600 nm reached 0.6-0.8. Then IPTG was added to a final concentration of 1mM and the culture was incubated for an additional two hours. The cells were harvested by centrifugation at 5,000g for 10 minutes at room temperature. The pellet was stored at -20°C.

2.6.2. Purification

Purification was performed according to the method described in Norris and Li, 1997.

a. Lysis

The frozen pellet was thawed at RT and resuspended in 1-2 pellet volumes of freshly prepared lysis buffer (50 mM Tris-HCl, pH 8, 10%(w/v) sucrose, 1 mM EDTA, 0.05%(w/v) sodium azide, 0.5 mM DTT, 10 mM $MnCl_2$, 2.5 mM phenylmethylsulfonyl fluoride, 2.5 mM benzamidine, 6µg/mL DNase). The lysate was incubated for 30 mins. at room temperature. Further lysis was accomplished by sonication (six 30-sec bursts at 10,000 psi alternated with five 30-sec delay on ice). The insoluble fraction of the lysate was removed by centrifugation at 15,000 rpm 1 hour at 4°C and the supernatant is stored at -20°C.

b. Gel filtration on Sephacryl S300

The supernatant from step a. was loaded onto a 2.5x100 cm column of sephacryl S-300 (Pharmacia) at room temperature. The elution buffer was 20 mM Tris-HCl, pH 8, 0.5mM DTT, 0.05% sodium azide (buffer A). The flow rate was 2mL/min. Collected 2-mL fractions were checked for ultraviolet absorbance at 280 nm. The fractions representing the peaks were examined by SDS-page for the presence of CRABPII.

c. Anion-exchange chromatography

2 mL of gel filtration fractions containing CRABPII were loaded onto a quaternary amine anion-exchange column FPLC (Fast Protein Liquid Chromatography) type monoQ (Pharmacia). CRABPII was eluted using a gradient buffer from 100% buffer A to 30% buffer B (100 % buffer B = buffer A + 250 mM NaCl) over a 20-min period at room temperature. 1 mL-fractions were collected every minute. Once more, the presence of CRABPII was checked by SDS page. CRABPII was stored at 4°C before freeze-drying using a Micromodulyo 1.5K with vial.

platform attachment (Edwards High Vacuum International). The desiccated samples were stored at room temperature until their use in the binding assay.

d. Detection of the presence of CRABPII

The expression and purification of CRABPII was validated using denaturing SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis on a 7-15% polyacrylamide gel (Biorad). 10 µL samples were mixed with 10 µL of 2X loading buffer (100 mM Tris-HCl pH6.8, 4% SDS, 0.2% BPB, 20% glycerol, 1mM DTT) and denatured by heating (2 mins. at 80°C). The samples were loaded onto the gel that was immersed in a 1X Tris-glycine buffer (Biorad) and a constant current (25 mA) was applied for 1 hour at room temperature. After Coomassie blue staining, the protein was identified according to its molecular weight as determined with the Benchmark pre-stained protein ladder (Gibco BRL).

A western blot was used to confirm the presence of CRABPII. The proteins separated on the SDS-PAGE were transferred on an Immobilon-P transfer membrane (Millipore) using a Biorad cassette. The transfer occurred in 1X Tris-glycine buffer (Biorad) + 10% methanol. An electrical currant (60 mA) was applied for 3 hours to allow the protein to migrate through the membrane. Afterwards, the membrane was blocked with 5% dry milk in 1X TBS for one hour at room temperature and probed with primary antibodies to CRABPII (1/1000 dilution of mouse anticlonal 5-CRA-B3) in the same buffer at 4°C overnight. The following day, the membrane was washed with PBS (3 x 5 minutes) and then incubated with 1:2000 dilution of the secondary antibody, peroxidase conjugated anti-mouse

antibody (ECLTM, Amersham), for 1 hour at room temperature. The membrane was washed with 1xPBS (3x5 minutes) and the protein was detected using ECL detection kit according to the manufacturer instruction (Amersham).

The concentration of purified CRABPII was determined using BSA kit (Pierce).

2.6.3. Radioactive Binding assay

220 pmol of CRABPII was incubated in 20 mM Tris-HCl buffer pH 7.4 with 15 pmol of radioactive all trans retinoic acid (NEN) in a total volume of 70µL. For the competitive assay, another ligand in excess (6670:1, 670:1 or 70:1) was added to the mix. The reaction occurred for one hour at room temperature in the dark. In order to separate the unbound all-trans retinoic acid from the bound all-trans retinoic acid, a 6kD cut-off minichromatography column (Biorad) was used. The storage buffer was discarded using a Microplex manifold for according to the manufacturer instruction (Pharmacia). The samples were loaded onto the column and the separation occurred by gravity over a 30-min period. Retinoic acid ("RA") bound to CRABPII appeared in the filtrate while free RA remained in the column. The radioactivity of the filtrate was measured by scintillation counter.

2.7 Assay for NADPH dependent retinoic acid oxidation (To identify B5)

The procedure below is a modification of a method described in the literature (4). The following assay buffer was

prepared and stored at 4°C: 0.1M PO_4 / 0.1mM EDTA / 5mM $MgCl_2$, pH 7.4. On the day of the assay, a 60mM NADPH solution in buffer was prepared. Inhibitor stocks, acidified ethanol / BHT quench solution, and hexane / BHT were prepared as described above. A working 1mM retinoic acid solution was prepared by dilution of a 15mM stock (in DMSO) with ethanol.

To a 2 dram vial, the following were added in order: assay buffer to give a final volume of 500µL, 20µL 60mM NADPH, 5µL inhibitor or solvent blank, followed by approximately 2mg of rat liver microsomal protein.

The mixture was incubated for 5 mins. at 37°C, then 5µL working 1mM retinoic acid solution was added. Incubation was continued for 60mins. at 37°C - the vials were not capped, since the oxidation process required molecular O_2 in addition to NADPH. Quenching was carried out with acidified ethanol/BHT and extraction was carried out with hexane/BHT as described above. Quantitation of the quickly eluting polar retinoic acid metabolites (presumed to be 4-oxo retinoic acid) was carried out by integration of the HPLC signal as described below.

All steps subsequent to the addition of retinoic acid were done in the dark or under amber lights. The final incubation solution contained 2.4mM NADPH, 100µM or less inhibitor, 10µM retinoic acid, approximately 4mg/mL rat liver microsomal protein and nearly 0.1M PO_4 / 0.1mM EDTA / 5mM $MgCl_2$.

HPLC analysis of individual retinoids

Samples for retinoid quantitation by HPLC were prepared by dissolving the residue in each vial with 100µL of methanol. The solution was transferred to a 150µL glass conical tube within a 1mL shell vial, capped tightly, and placed inside a Waters 715 Autosampler. Aliquots of 60µL were injected immediately and analysed for retinoid content.

The chromatography instrumentation consisted of a Waters 600 gradient controller/pump, a Waters 996 Photodiode Array detector and a Waters 474 Scanning Fluorescence detector. Two HPLC protocols were used for retinoid analysis. For the ARAT and LRAT assay, the separation of retinol and retinol esters was performed with a Waters 3.9x300mm C18 Novapak reverse-phase analytical column and Waters Sentry NovaPak C18 guard column with an 80:20(v/v) methanol/THF isocratic mobile phase adjusted to a flow rate of 1mL/min. for 10 min. The eluate was monitored for absorbance at 325nm and fluorescence at 325ex/480em.

A shorter Waters 3.9x150mm C18 Novapak reverse-phase analytical column and Waters Sentry NovaPak C18 guard column were used to separate retinoid acids and alcohols for the retinol and retinoic acid oxidation assays utilising a modification of a gradient system described by Barua (5). This system consisted of a 20 mins. linear gradient from 68:32(v/v) methanol/ water containing 10mM ammonium acetate to 4:1(v/v) methanol:dichloromethane followed by a 5 mins. hold at a flow rate of 1mL/min. The column eluate was monitored from 300nm to 400nm.

These protocols were selected based on their ability to clearly resolve pertinent retinoid acids, alcohols, aldehydes, and/or esters for each assay and relative quickness of separation. Identification of individual retinoids by HPLC was based on an exact match of the retention time of unknown peaks with that of available authentic retinoid standards and UV spectra analysis (300-400nm) of unknown peaks against available authentic retinoids.

References

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The boosters suitable for use in the present invention include but are not limited to the boosters listed in Tables B_1 through to B_5 below. The table below gives the booster

class (B_1 - B_5), the chemical name of the compound, and the results from the appropriate assays used to identify the booster (i.e. ARAT/LRAT for B1, retinol dehydrogenase for B_2, retinaldehyde inhibation for B3, CRABP is binding for B_4 and retinoic acid oxidation inhibition for B_5.

ARAT/LRAT Inhibitors (B1)

Class	Compound	%Inhibition Overall TG(-ROH/RE)	Overall TG (IC 50)	%Inhibition ARAT(10μm)	%Inhibition ARAT (100μm)	%Inhibition LRAT (10μm)	%Inhibition LRAT(100μm)
Carotenoid	Crocetin		3.75E-05	15%	34%	0	15%
Fatty Acid & Other Surfactants	Acetyl Sphingosine		6.78E-06	19%+/-12	62%+/-11	10%+/-10	50%+/-18
Fatty Acid Amides & Other Surfactants	C13 Beta-Hydroxy Acid/ Amide	17%			28%		25%
Fatty Acid Amides & Other Surfactants	Castor Oil MEA		3.25E-05				
Fatty Acid Amides & Other Surfactants	Cocamidopropyl Betaine				25%		
Fatty Acid Amides & Other Surfactants	Coco Hydroxyethyl-imidazoline		2.84E-07		68%		65%
Fatty Acid Amides & Other Surfactants	Cocoamide-MEA (or Cocoyl Monoethanol-amide)	11%			13%		34%
Fatty Acid Amides & Other Surfactants	Glycerol-PCA-Oleate				41%+/-6		58%+/-2
Fatty Acid Amides & Other Surfactants	Hexanoamide				20%		
Fatty Acid Amides & Other Surfactants	Hexanoyl Sphingosine		9.99E-05		28%+/-4		37%+/-9
Fatty Acid Amides & Other Surfactants	Hydroxyethyl-2-Hydroxy-C12 Amide		3.29E-05		35%		35%
Fatty Acid Amides & Other Surfactants	Hydroxyethyl-2-Hydroxy-C16 Amide				25%		30%
Fatty Acid Amides & Other Surfactants	Lauroyl Sarcosine				20%		
Fatty Acid Amides & Other Surfactants	Lidocaine				12%		0
Fatty Acid Amides & Other Surfactants	Linoleamide-DEA (or Linoleoyl Diethanolamide)	59%		12%+/-3	43%+/-3	11%+/-9	51%+/-15
Fatty Acid Amides & Other Surfactants	Linoleamide-MEA (or Linoleoyl Monoethanol-amide)		1.61E-05	14%	35%	20%+/-8	35%
Fatty Acid Amides & Other Surfactants	Linoleamidopropyl Dimethylamine				69%+/-18		75%+/-4
Fatty Acid Amides & Other Surfactants	Melinamide				64%+/-15		43%+/-21
Fatty Acid Amides &	Myristoyl Sarcosine				41%+/-14		11%+/-11

Other Surfactants							
Fatty Acid Amides & Other Surfactants	Oleyl Betaine		2.80E-05		47%		
Fatty Acid Amides & Other Surfactants	Palmitamide-MEA			6%	23%	12%	33%
Fatty Acid Amides & Other Surfactants	Stearylhydroxyamide				10%		10%
Fatty Acid Amides & Other Surfactants	Utrecht-1	21%		43%	54%	51%	48%+/-6
Fatty Acid Amides & Other Surfactants	Utrecht-2		3.47E-06	42%	83%+/-9	51%	92%+/-3
Flavanoids	Naringenin				33%		14%
Fragrances	Allyl Alpha-Ionone			16%+/-14	22%+/-23	17%+/-10	36%/-7
Fragrances	Alpha-Damascone		3.35E-04	67%+/-27	83%+/-12	87%+/-6	98%+/-1
Fragrances	Alpha-Ionone		9.27E-04		45%+/-27		49%+/-30
Fragrances	Alpha-Methyl Ionone				67%		77%
Fragrances	Alpha-Terpineol				26%		25%
Fragrances	Beta-Damascone			45%	84%	52%	92%
Fragrances	Brahmanol				70%		75%
Fragrances	Damascenone			23%	70%	29%	79%
Fragrances	Delta-Damascone			58%	87%	64%	95%
Fragrances	Dihydro Alpha-Ionone				13%		18%
Fragrances	Ethyl Saffranate				51%		49%
Fragrances	Fenchyl Alcohol				12%		4%
Fragrances	Gamma-Methyl Ionone				21%		38%
Fragrances	Isobutyl Ionone				8%		45%
Fragrances	Isocyclogeraniol				18%		16%
Fragrances	Isodamascone				80%		92%
Fragrances	Lyral		1.27E-04		76%		71%
Fragrances	Santalone				23%		12%
Fragrances	Santanol				15%		43%
Fragrances	Timberol				34%		33%
Fragrances	Tonalid				50%		33%
Fragrances	Traseolide				41%		21%
Miscellaneous	Coco Trimethyl-ammonium Cl-				27%		
Miscellaneous	Urosolic Acid		1.46E-06		21%		28%
Noncyclic Fragrances	Citral				20%		

Noncyclic Fragrances	Citronellol			30%		0
Noncyclic Fragrances	Farnesol	9.35E-05	23%+/-18	53%+/-18	10%+/-7	53%+/-19
Noncyclic Fragrances	Geraniol	7.83E-03	13%	32%		
Noncyclic Fragrances	Geranyl Geraniol		38%+/-12	81%+/-6	16%+/-9	77%+/-13
Noncyclic Fragrances	Linalool			28%		0
Noncyclic Fragrances	Nonadieneal			20%		
Noncyclic Fragrances	Pseudolonone			12%		37%
Phospholipid	Dioctylphosphatidyl Ethanolamine		23%	50%+/-2	0	17%+/-17
Urea	Dimethyl Imidazolidinone	22%				
Urea	Imidazolidinyl Urea	35%				

Retinol Dehydrogenase Activators (B2)

Class	Compound	%Increase Retinol Dehydrogenase
Phospholipid	Phosphatidyl Choline	21% increase
Phospholipid	Sphingomyelin	26% increase

Retinaldehyde Reductase Inhibitors (B3)

Class	Compound	Overall TG(IC 50)	% Inhibition Retinal Reductase
Aldehyde	Vanillin	9.70E-03	6%
Fatty Acid	Arachidic Acid		20%
Fatty Acid	Arachidonic Acid		49%
Fatty Acid	Linoleic Acid	1.63E-04	62%+/-2
Fatty Acid	Linolenic Acid	1.34E-04	54%+/-16
Fatty Acid	Myristic Acid	1.72E-05	26%
Miscellaneous	Amsacrine	6.26E-06	22%+/-8
Miscellaneous	Carbenoxolone	3.61E-07	26%+/-2
Miscellaneous	Glycyrretinic Acid	8.64E-06	38%+/-1
Phospholipid	Phosphatidyl ethanolamine		37%

CRABPII Antagonists (B4)

Class	Compound	Overall TG(IC 50)	% Inhibition CRABPII
Fatty Acid	Elaidic Acid	6.50E-05	>50%
Fatty Acid	Hexadecanedioic Acid	1.30E-04	>50%
Fatty Acid	12-Hydroxystearic Acid	2.91E-05	>50%
Fatty Acid	Isostearic Acid	6.88E-05	>50%
Fatty Acids	Linseed Oil		>50%

Retinoic Acid Oxidation Inhibitors (B5)

Class	Compound	Overall TG(IC 50)	%Inhibition Retinoic Acid (10μM)	% Inhibition Retinoic Acid(100μM)
Imidazole	Bifonazole		89%	100%
Imidazole	Climbazole	4.47E-06	80%	92%
Imidazole	Clotrimazole		76%	85%
Imidazole	Econazole		88%	100%
Imidazole	Ketoconazole	1.85E-07	84%	84%
Imidazole	Miconazole	2.78E-07	74%	86%
Fatty Acid Amides & Other Surfactants	Lauryl Hydroxyethylimidazoline	4.67E-07		
Fatty Acid Amides & Other Surfactants	Oleyl Hydroxyethylimidazoline	3.02E-05	54%	80%
Flavanoids	Quercetin	6.29E-05	40%	74%
Coumarin	Coumarin			
Quinoline	(7H-Benzimidazo [2,1-a]Benz [de]-Isoquinolin-7-one	8.59E-07		
Quinoline	Hydroxyquinoline (Carbostyril)	3.64E-04		
Quinoline	Metyrapone (2-Methyl-1,2-di-3-Pyridyl-1-Propane)			47%

SECTION B. Effects Of Booster Combinations

In order to assess the effect of combinations of booster molecules an assay is required which encompasses the effect of each of the five booster classes. A single enzyme assay is not suitable for this purpose, as it will be specific only for one class of booster molecule. An assay which reflects retinoid concentration in keratinocytes is necessary to relate the effects of single booster molecules with combination of booster molecules. For this reason, a transglutaminase (Tgase) assay was utilised. Tgases are calcium dependent enzymes that catalyse the formation of covalent cross-links in proteins. Several Tgase enzymes are membrane bound in keratinocytes which is important for epidermal cell maturation. This enzyme is inhibited by retinoic acid. The higher the concentration of retinoic acid, the greater the inhibition of Tgase expression. Hence Tgase is a good marker of both keratinocyte differentiation and of the retinoid effect on keratinocytes.

Transglutaminase as a marker of skin differentiation

During the process of terminal differentiation in the epidermis, a 15nm thick layer of protein, known as the cornified envelope (CE) is formed on the inner surface of the cell periphery. The CE is composed of numerous distinct proteins which have been cross-linked together by the formation of Nε-(γ-glutamyl) lysine isodipeptide bonds catalysed by the action of at least two different

transglutaminases (TGases) expressed in the epidermis. TGase I is expressed in abundance in the differentiated layers of the epidermis, especially the granular layer, but is absent in the undifferentiated basal epidermis. Thus TGase I is a useful marker of epidermal keratinocyte differentiation with high TGase I levels indicating a more differentiated state. An ELISA based TGase I assay, using a TGase I antibody, was used to assess the state of differentiation of the cultured keratinocytes in the examples that follow.

Keratinocytes (cultured as described above) were plated in 96 well plates at a density of 4,000-5,000 cells per well in 200µl media. After incubation for two to three days, or until cells are ~50% confluent, the media was changed to media containing test compounds (five replicates per test). The cells were cultured for a further 96 hours after which time the media was aspirated and the plates stored at -70°C. Plates were removed from the freezer, and the cells were washed twice with 200µl of 1xPBS. The cells were incubated for one hour at room temperature (R/T) with TBS/5% BSA (wash buffer, bovine serum albumin). Next the TGase primary antibody was added: 50µl of monoclonal anti-Tgase I Ab B.C. diluted 1:2000 in wash buffer. The primary antibody was incubated for 2 hours at 37°C and then rinsed 6x with wash buffer. Cells were then incubated with 50µl of secondary antibody (Fab fragment, peroxidase conjugated anti-mouse IgG obtaining from Amersham) diluted 1:4,000 in wash buffer for two hours at 37°C, then rinsed three times with wash buffer. Following the rinse with washing buffer, the cells were rinsed 3x with PBS. For colourimetric development, the cells

were incubated with 100µl substrate solution (4 mg o-phenylenediamine and 3.3 µl 30% H_2O_2 in 10ml 0.1M citrate buffer pH 5.0) for exactly five minutes, R/T, in darkness (under aluminum foil). The reaction was stopped by the addition of 50µl 4N H_2SO_4. The absorbance of samples was read at 492nm in a 96 well plate UV spectrophotometer. Out of the five replicates, four were treated with both antibodies, the fifth one was use as a Tgase background control. TGase levels were determined and expressed as percentage control.

Details of of Tgase assay:

Prior to initiating experiments, to determine the effects of combinations of booster molecules standard Tgase assay conditions were investigated. A fully validated Tgase assay was established as follows:

A. Reagents

Cells: Human Keratinocytes (P2 in T75 flasks; P3 in 96 well assay plates)	Neonatal Human foreskin
Primary Antibody: TGm specific monoclonal Ab B.C1	Biogenesis (Cat# 5560 -6006)
Secondary Ab: Peroxidase labeled antimouse Ig F(ab)2	Amersham (Cat # NA9310)
Substrate solution: For 10 ml phosphate citrate buffer	
4.0 mg o-phenylenediamine	Sigma P-7288
3.3 µl of 30% H_2O_2	Sigma H-1909

B. Media/Buffers

Keratinocyte Growth Media (KGM)	Clonetics (Cat# 3111)
Phosphate Buffered Saline; Dulbecco's without $Ca/MgCl_2$)	Life Technology (Cat # 14200-075)
Tris Buffered Saline	
Blocking buffer (1xTBS + 5% dry milk)	BioRad (Cat #170-6404)
Washing buffer (1% dry milk in TBS + 0.05% Tween 20)	Sigma (Cat # P-7949)
Phosphate citrate buffer: 1:1 mixture of 0.2M dibasic sodium phosphate and 0.1 M citric acid	Sigma (Cat # S-9763) Sigma (Cat # C-1909)
4 N H_2SO_4	

C. Culture ware

96-well polypropylene microtitre plate	Costar (Cat # 3595)
96-well polypropylene U-bottom plate	Costar (Cat # 3794)
T75- vent cap	Costar (Cat # 3376)

D: Instrumentation/Equipment

Biotek Model EL 340 Microplate reader	Bio-tek Instuments Inc.
Multiprobe II	Packard

E: Cell Culture Procedure

Seeding of Keratinocytes in 96 well plates

1. A suspension of keratinocytes was prepared at a concentration of 3000 cells/200 µl/ well in KGM medium (Used $3x10^5$ cells /12 ml media in each microtitre plate)
2. 200µl of the keratinocyte suspension was transferred into each of the inner 60 wells only.
3. 200µl of KGM media was pipetted into the outer wells (to maintain thermal equilibrium).
4. Each plate was incubated at 37°C and 5% CO_2 for 3 days or until cells are ~50% confluent.

Treatment of keratinocytes with samples.

5. Stock solutions of the samples were prepared in DMSO.
6. The samples were diluted to desired concentration with the final assay concentration of DMSO being 0.1 %.
7. 20 µl of the sample was transferred into wells and 180 µl of KGM medium added to give a final assay volume of 200 µl.
8. Plates were incubated at 37°C and 5% CO_2 for 72 hours.
9. Media were completely removed from each well.
10. Wells were rinsed with 2x with 200 µl of 1xPBS
11. Finally they were frozen for at least 1.5 hours at -70°C.

F: Transglutaminase Assay

1. Block:

Incubate plates at room temperature with 200 µl/well of blocking buffer for 1 hour.

2. Primary Antibody:

Aspirate blocking buffer. Incubated with 100 µl/well of TGm-specific monoclonal antibody B.C1 (diluted 1:2000 in washing buffer) at 37°C for at least 2 hours. The primary antibody was not added in background control wells.

3. Rinsed wells 6x with washing buffer.

4. Secondary Antibody:

Incubated with 100 µl/well peroxidase labeled anti-mouse IgF(ab)2 fragment (diluted 1:4000 in washing buffer) at 37°C for 2 hours.

5. Rinsed wells 3X with washing buffer (added 200µl) and aspirated after each rinse.

6. Rinsed wells 3X with PBS w/o Tween.

7. Incubated with 100 µl/well substrate solution at room temperature for exactly 5 minutes.

8. Stopped reaction with 50 µl/well 4N H_2SO_4.

9. Read absorbance at 492 nm in the Bio-tek plate reader.

I. Optimization Studies

a. Time Course of Transglutaminase Production

A time course experiment was conducted to determine the optimal incubation time for transglutaminase production in keratinocytes grown in 96-well plates (4000

cells/well). This time course study was conducted with multiple variables including dose response analyses of retinoic acid and retinol as well as incubation in the presence of 1.2 mM $CaCl_2$. Although the transglutaminase production in the control cells (0.1% DMSO) was not altered, both retinoic acid and retinol exhibited a dose dependent inhibition of transglutaminase production over the five day incubation period. The most pronounced retinoid effect was observed on day 2 and day 3. The maximal inhibition was observed on day 2 with the transglutaminase production being inhibited by 85% and 55% in the presence of the highest concentration (1 µM) of retinoic acid and retinol respectively. The same experiment was also conducted with varying cell density (3000 cells/well and 5000 cells/well) and comparable results were observed.

B: DMSO Sensitivity

Various concentrations of DMSO ranging from 0-2% were tested for the effect on transglutaminase production in keratinocytes. The assay was sensitive to DMSO concentration with significant inhibition of activity, above 0.5% DMSO. Hence, a final assay concentration of 0.1% was selected for subsequent sample concentration studies.

C: Dose Response Curves: Retinoic Acid and Retinol

Based on the data, day 3 was selected as the optimal time and 0.1%DMSO was selected as the concentration to be used for further testing. An additional dose

response experiment was carried out with retinoic acid and retinol in the presence of 0.1% DMSO, with the transglutaminase production being assayed on day 3. A good dose response was observed for Tgase inhibition by retinoic acid and retinol. 10-7M retinol gave an inhibition of Tgase in the linear range of concentration. Therefore, this concentration of retinol was chosen to evaluate the booster combinations.

D: Final conditions used to test boosters or combination of boosters

Days of incubation of keratinocytes with retinol and boosters	-	3 days
Final DMSO concentration	-	less than 0.1%
Retinol concentration	-	10-7M (0.1µM)
Booster concentrations	-	10 mM to 0.1 nM

Using the above conditions, dose response for all the different boosters (B1-B5) were tested to identify the best concentration of booster to test in combinations.

Transglutaminase levels were determined and expressed in the Tables B1 through B5 either as:

(i) % (booster + retinol inhibition / control inhibition) - % (ROH inhibition / control inhibition), which measures the added effect of booster + retinol induced TGase inhibition over retinol alone, or

(ii) as an IC50 value when the inhibitory effect of multiple booster concentrations was examined - this provides the concentration of booster which, in combination with a

constant retinol concentration of 10^{-7} M, inhibits TGase by 50%.

Booster combinations and booster ratios:

It has been discovered surprisingly that certain compounds increase the endogenous levels of retinoic acid formation from retinol or retinyl esters by different mechanisms. These compounds are collectively called here as "retinoid boosters". These include: inhibitors of ARAT/LRAT (B1 boosters), inhibitors of retinaldehyde reductase (B3 boosters), inhibitors of retinoic acid binding to CRABP-2 (B4 boosters) and inhibitors of retinoic acid oxidation catalysed by cytochrome P450 enzymes (B5 boosters), or certain other compounds which enhance or activate retinol dehydrogenase (B2 boosters). These boosters are coded as groups B1 through to B5, as seen in chart 1 herein above.

The boosters alone or in combination with each other, potentiate the action of a retinoid by increasing the amount of retinol available for conversion to retinoic acid and inhibiting the degradation of retinoic acid. The boosters act in conjunction with a retinoid (e.g. retinol, retinyl ester, retinal, retinoic acid) the latter being present endogenously in the skin. The preferred compositions, however, include a retinoid in the composition, co-present with a booster, to optimise performance.

The present invention includes, in part, a second composition containing from about 0.0001% to about 50%,

preferably from 0.001% to 10%, most preferably from 0.001% to 5% by weight of the composition of at least one booster compound, or a combination of binary, tertiary, quaternary or 5 booster combinations. The combined concentration of the booster combinations of 0.001% to 5% in specified ratios as shown below, inhibit transglutaminase in an in vitro transglutaminase assay to more than 50%, and a cosmetically acceptable vehicle.

The boosters included in the inventive compositions are selected from the group consisting of:

a. Two boosters, wherein both are selected from the group consisting of B2, B3 and B4;
b. Binary combinations of boosters selected from the group consisting of B1/B2; B1/B3, B1/B4; B1/B5; B2/B3, B2/B4; B2/B5; B3/B4, B3/B5; B4/B5
c. Ternary combinations of boosters selected from the group consisting of B1/B2/B3;B1/B2/B4;B1/B2/B5; B1/B3/B4;B1/B3/B5; B1/B4/B5; B2/B3/B4; B2/B3/B5; B2/B4/B5;B3/B4/B5
d. Quaternary combinations of boosters selected from the group consisting of B1/B2/B3/B4; B1/B2/B3/B5; B1/B2/B4/B5; B1/B3/B4/B5; B2/B3/B4/B5; and
e. A combination of five groups of boosters B1/B2/B3/B4/B5.

Booster to booster ratios:

The boosters of different classes (B1 to B5) in combinations as shown above have an optimal concentration of between 0.001% to 5% in a cosmetic product at specific ratios as

shown below for inhibition of Tgase activity to at least below 50%:

Invention	Ratios of boosters to boosters	Concentrations
Broad	1: 10,000 to 10,000:1	100 mM to 1 nM
Preferred	1: 1000 to 1000:1	10 mM to 10 nM
Most preferred	1:100 to 100:1	1 mM to 100 nM
Optimum	1:10 to 10:1	0.1 mM to 1 μM

Retinoid to booster ratios:

The preferred composition includes a retinoid (e.g.retinol, retinyl ester, and retinaldehyde) in the composition, co-present with a booster or a combination of the boosters, to optimise performance.

For optimum performance, the concentration of retinoid to booster should be present in the composition in ratios as given below:

Invention	Ratios of boosters to retinoids	Concentrations
Broad	10,000:1 to 1:10,000	100 mM- 1 nM booster; 0.001-10% retinoids
Preferred	1000:1 to 1:1000	10 mM-10 nM booster; 0.001-10% retinoid
Most preferred	100:1 to 1:100	1 mM-100 nM booster; 0.01-1% retinoid

Concentrations of individual boosters used in the examples:

Since the objective is to establish synergistic inhibition of transglutaminase expression by combinations of the active compounds with retinol, it was essential to determine the dose response profiles (IC_{20} and IC_{50} values) of the active compounds, when tested individually in the presence of

retinol. The detailed dose response of boosters belonging to B2-B4 is given in the tables following the IC50 and IC 20 table below. This data was used to identify an appropriate sub-maximal inhibitory concentration of each active compound, to eventually make it possible to identify putative synergistic effects of the mixtures of the active compounds in the presence of retinol. The data in the following table represents the IC_{50} and IC_{20} (80% of control) values and the concentrations used when testing synergies with combinations of boosters.

In order to demonstrate synergy of two compounds, it is essential to select concentrations to test that are at most IC20, in other words, a compound concentration that individually boosts the retinol inhibition of Tgase expression by 20%. Two such compounds should have an additive inhibition of 40%. Using this strategy to determine concentrations leaves a window of 40-100% for further inhibition for detecting synergy of the two compounds under examination.

A more challenging concentration criterion would be selecting concentrations of compounds which alone showed no inhibition effect, but in combination show inhibition. In this study however, we chose an even more challenging criteria. We selected concentrations of compounds that were 10 to 1000 fold lower than the minimally effective Tgase inhibiting concentration. Identification of synergistic combinations using such very low concentrations would mean

that the most effective synergistic combinations were identified.

Booster Class	Compound Name	IC_{50}	IC_{20}	Con. Used for synergy (binary, tertiary, quaternary)
B1	LinoleoylMonoethanolamide (LAMEA)	1.61E-05	1.48E-05	1E-05 to 1E-09
	Palmitamide Monoethanolamide	ND	ND	1E-06 to 1E-10
	Oleyl Betaine	2.80E-05	1.08E-05	1E-05 to 1E-8
	Naringenin	ND	ND	1E-05 to 1E-09
	Echinacea	ND	ND	1E-05 to 1E-09
	Dimethyl imidazolinone	ND	ND	1E-05 to 1E-09
	Melinamide	ND	ND	1E-05 to 1E-09
	Geranyl geraniol	ND	ND	1E-05 to 1E-09
	Farnesol	9.35E-05	7.82E-05	1E-06 to 1E-09
	Geraniol	7.83E-03	4.72E-03	1E-03 to 1E-07
	α-Damascone	3.35E-04	1.69E-04	1E-04 to 1E-08
	α -Ionone	9.27E-04	1.42E-04	1E-04 to 1E-08
	Castor oil Methyl Ester Acid (MEA)	3.25E-05	9.38-E06	1E-06 to 1E-09
	Ursolic Acid	1.46E-06	5.94-E07	1E-06 to 1E-09
	Utrecht-2	3.47-E06	3.30-E06	1E-06 to 1E-09
	Cocoyl hydroxyethylimidazoline	2.84E-07	9.21E-08	1E-08 to 1E-11
	Acetyl sphingosine (C2 Ceramide)	6.78E-06	5.15E-06	1E-06 to 1E-09
	Hexanoyl sphingosine (C6 Ceramide)	9.99E-05	6.94E-05	1E-05 to 1E-09
	Crocetin	3.75E-05	2.52E-05	1E-05 to 1E-09
	Lyrial	1.27E-04	4.00E-05	1E-05 to 1E-09
	N-Hydroxyethyl-2-hydroxydodecyl amide	3.29E-05	2.40E-05	1E-05 to 1E-09
B2	Phosphatidyl Choline	ND	ND	1E-05 to 1E-09
	Sphingomyelin	ND	ND	1E-05 to 1E-09
	TCC	9.64E-07	6.18-E07	1E-07 to 1E-10
	1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	ND	ND	1E-05 to 1E-09
B3	Amsacrine-HCl	6.26E-06	3.30E-06	1E-06 to 1E-09
	Carbenoxolone	3.61E-07	2.00E-07	1E-07 to 1E-10
	Glycyrrhetinic Acid	8.64E-06	5.96E-06	1E-06to 1E-09
	Linoleic Acid	1.63E-04	8.95E-05	1E-05 to 1E-09
	Linolenic Acid	1.34E-04	1.21E-04	1E-05 to 1E-09
	Arachidonic Acid (Na+ salt)	ND	ND	1E-05 to 1E-09
	Myristic Acid	1.72E-05	1.05E-05	1E-05 to 1E-09
	Vanilin	9.70E-03	8.47E-03	1E-03 to 1E-06
B4	Hexadecanedioic acid	1.30E-04	8.40E-05	1E-05 to 1E-09
	12-Hydroxystearic acid	2.91E-05	1.45E-05	1E-05 to 1E-09
	Elaidic acid	6.50E-05	5.88E-05	1E-05 to 1E-09
	Linseed oil	ND	ND	1E-05 to 1E-09
	Isostearic acid	6.88E-05	6.23E-05	1E-05 to 1E-09
	2-Hydroxystearic acid	ND	ND	1E-05 to 1E-09
B5	Climbazole	4.47E-06	2.45E-07	1E-07 to 1E-10

	Clotrimazole	ND	ND	1E-05 to 1E-09
	Miconazole	2.78E-07	8.42E-08	1E-08 to 1E-11
	Coumarin	ND	ND	1E-05 to 1E-09
	Ketoconazole	1.85E-07	5.52E-08	1E-08 to 1E-11
	3,4,-Dihydro-2(1H)-quinolinone(Hydrocarbostyril)	ND	ND	1E-05 to 1E-09
	2-Hydroxyquinoline(Carbostyril)	3.64E-04	1.70E-04	1E-04 to 1E-08
	Amino Benzotriazole	ND	ND	1E-05 to 1E-09
	Lauryl hydroxyethylimidazoline	4.67E-07	2.69E-07	1E-07 to 1E-10
	Quercetin	6.29E-05	5.11E-05	1E-05 to 1E-09
	Oleoyl hydroxyethlimidazoline	3.02E-05	5.65E-06	1E-06 to 1E-09
	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	8.59E-07	4.69E-07	1E-07 to 1E-09

ND: Not determined or a clear dose response was not observed. For synergies, a wide range of concentration (4 orders of magnitude 10-5 to 10-9M) was tested.

Dose response for boosters class B2 to B4

The following tables include the data on the dose response of boosters belonging to class B2 to B4. Concentration of boosters are given in Molar; mean Tgase level and Standard deviation of 4 replicates is expressed as % of control (0.1% DMSO and 10-7M retinol). Higher numbers (close to 100 or above 100) indicate no inhibition of Tgase. The lower the number, the more potent the inhibitor is at that concentration. The IC50 and IC20 values were calculated from this dose response table and expressed in the above table.

B2 class boosters:

Phosphatidyl choline (B2)

Concentration	Tgase levels (Mean)	Tgase (SD)
4.4E-05	90.9	0.01
1.47E-05	120.3	10.6
4.89E-06	70.1	11.4
1.63E-06	98.8	0.00
5.43E-07	86.7	6.19
1.8E-07	75.9	20.5
6.0E-08	87.8	3.9
1.2E-08	159	42.3
2.4E-09	85.5	0.39

Sphingomyelin (B2)

Concentration	Tgase levels (Mean)	Tgase (SD)
3.0E-05	45	3.21
1.0E-05	77.8	25.5
3.33E-06	76.4	7.55
1.1E-06	98.8	0.00
3.73E-07	91.6	14.9
1.23E-07	70.0	3.63
4.10E-08	74.6	4.19
8.2E-08	115.2	1.02
1.65E-09	68.4	2.03
3.29E-10	69.2	2.1

TCC (B2)

Concentration	Tgase levels (Mean)	Tgase (SD)
1.14E-03	36.3	4.6
3.8E-04	3.8	0.96
3.31.23E-04	-3.2	0.91
4.22E-05	-11.2	0
1.41E-06	-.3	4.88
4.69E-07	15.9	3.52
6.26E-08	18.9	3.12
1.25E-08	100.2	23.3
6.9E-09	77.6	21.2
1.0E-09	54.4	11.23

5

1,2 dioctanoyl-sn-glycero-3-phopshoethanolamide (B2)

Concentration	Tgase levels (Mean)	Tgase (SD)
1.6E-04	58.1	2.08
5.33E-05	95.4	21.3
1.78E-05	104	4.01
5.93E-06	129	0.0
1.98E-06	110	8.74
6.58E-07	92.8	15.78
2.19E-09	88.6	12.3
4.39E-08	127.3	3.39
8.78E-09	119	21.1
1.79E-9	82	15.6

B3 Class boosters

Amscrine B3

Concentration	Tgase levels (Mean)	Tgase (SD)
3.0E-05	-10	3.29
1.0E-05	1.8	7.45
3.33E-06	64	4.2
1.1E-06	84	0
3.73E-07	109	6.2
1.23E-07	65	15.8
4.10E-08	110	10.5
8.2E-08	131	27
1.65E-09	113	18
3.29E-10	92	8.9

Carbenoxolone (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
3.0E-06	-7.1	0
1.0E-06	27.3	1.15
3.33E-07	51.7	0
1.1E-07	158	0
3.73E-08	126	4.67
1.23E-08	81	29
4.10E-09	135	6.88
8.2E-10	112	32
1.65E-10	77.8	10.6
3.29E-11	64	49

Glyrrhetinic acid (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
3.0E-04	-0.3	3.9
1.0E-05	0.7	3.55
3.33E-05	2.5	2.1
1.1E-06	96.4	0.00
3.73E-06	120	33.2
1.23E-07	112	38
4.10E-07	93	11
8.2E-08	225	108
1.65E-08	103	11
3.29E-9	100	6.2

Linoleic acid (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
9.0E-03	-6	3.06
3.0E-03	0.1	2.01
1E-03	-16.4	16.3
1.1E-04	4.4	0
3.73E-04	79.2	0
1.23E-05	62.6	6.2
4.10E-05	76.8	3.69
8.2E-06	146	44.2
1.65E-07	106	20.2
3.29E-07	60.2	2.3

Linolenic acid (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
9.0E-03	-11	8.7
3.0E-03	-5.7	0.74
1E-03	-7.5	7.8
1.1E-04	-23	0
3.73E-04	68	0.57
1.23E-05	94.9	17.2
4.10E-05	65.9	0.03
8.2E-06	119	1.6
1.65E-07	77	8.5
3.29E-07	98	7.0

Myristic acid (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
1E-03	-2	4.1
1.1E-04	-8	2.3
3.73E-04	-6	1.16
1.23E-05		
4.10E-05	75.1	1.06
8.2E-06	74.2	10.0
1.65E-07	88.9	8.4
3.29E-07	101	4.47
5.0E-08		
1.1E-08		

Vanillin (B3)

Concentration	Tgase levels (Mean)	Tgase (SD)
1.4E-02	21.5	24.2
4.8E-03	93.8	1.7
1E-03	124	15.6
1.1E-04		
3.73E-04	101	14.3
1.23E-05	82	14.6
4.10E-05	98	2.4
8.2E-06	109	22
1.65E-07	80	4
3.29E-07	93	41

B4 Class boosters

Hexadecanedioic acid (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)
1E-03		
1.1E-04	14.2	2.7
3.73E-04	43.4	8.4
1.23E-05	130	0
4.10E-05	105	14
8.2E-06	114	12
1.65E-07	95	1.9
3.29E-07		
5.0E-08	74	6.7
1.1E-08	70	10.4

12-hydroxysteric acid (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)
3.73E-04		
1.23E-05	-5.2	2.3
4.10E-05	32.4	5.3
8.2E-06	97.6	0
1.65E-07	90.2	11
3.29E-07	82	28
5.0E-08	81	3.8
1.1E-08	98	24
2.0E-08	118	28
4.3E-09	71	2.3

Elaidic acid (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)
1E-03	12.8	12.1
1.1E-04	8	0.45
3.73E-04	13.8	1.92
1.23E-05	80.9	0
4.10E-05	58.2	8.8
8.2E-06		
1.65E-07	58	0.13
3.29E-07	69	44
5.0E-08	50.5	3.8
1.1E-08		

Linseed Oil (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)
1E-04	138	15
3.73E-05	145	2.5
1.23E-05	88	12
4.10E-06	113	0
8.2E-06	113	13
1.65E-07	96	18
3.29E-07	106	10
5.0E-08	134	22
1.1E-09	83	13
9.9E-10	73	15

Isosteric acid (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)
1E-03	-8.6	3.4
1.1E-04	1.2	3.0
3.73E-04	-5.3	1.1
1.23E-05	80	00
4.10E-05	67	7.9
8.2E-06	103	12.3
1.65E-07	95	5.5
3.29E-07	123	0.5
5.0E-08	78	12.2
1.1E-08	78	29

2-hydroxysteric acid (B4)

Concentration	Tgase levels (Mean)	Tgase (SD)
9.1E-04	46.6	6.2
3.73E-04	69.3	8.3
1.23E-04	51	8.8
3.10E-05	96.0	0.0
1.2E-05	105	30
3.65E-06	63	8.0
1.29E-06	80	4.7
2.0E-07	142	34
5.1E-08	64	20
1.0E-08	58	17

Synergy of Tgase inhibition with binary combinations of boosters

To investigate synergistic inhibition of Tgase expression by combinations of 2 different classes of boosters with retinol, selected combinations of compounds were tested at concentrations given in the above table. The concentrations tested were one log order of magnitude less than the concentration required for minimal inhibition of Tgase activity (i.e. IC_{20}). The compounds were tested alone and in combination and the % inhibition of Tgase is given for each compound and the combination.

The following examples give the synergistic combinations in all possible binary combinations (B1/B2; B1/B3, B1/B4; B1/B5; B2/B3, B2/B4; B2/B5; B3/B4, B3/B5; B4/B5). When the % inhibition of the combination is more than the inhibition of each compound added together, it indicates synergy (i.e. Inhibition by combination is greater than inhibition by

compound 1 + compound 2). All the binary combination examples given in the following table synergistically inhibited Tgase.

Binary combinations	Compound 1	Compound 2	TG as % C Compd 1	TG as % C Compd 2	TG % C Combination
B1/B2	Dimethyl imidazolidinone	Phosphatidylcholine	99	97	84
B1/B2	Alpha-demascone	Phosphatidylcholine	95	97	86
B1/B2	Hexanoyl sphingosine	Phosphatidylcholine	109	97	86
B1/B2	Alpha-ionone	Sphingomyelin	101	98	76
B1/B2	1,2 dioctanoyl-sn-glycero-3-phosphoethanolamide	Phosphatidyl choline	106	98	78
B1/B2	Alpha-demascone	Sphingomyelin	95	84	67
B1/B3	1,2 dioctanoyl-sn-glycero-3-phosphoethanolamide	Amsacrine	123	134	75
B1/B3	1,2 dioctanoyl-sn-glycero-3-phosphoethanolamide	Carbenoxelone	123	164	96
B1/B3	Caster oil MEA	Carbenoxelone	96	164	67
B1/B3	Utrecht-2	Amsacrine	102	98	86
B1/B3	Utrecht-2	Carbenoxelone	102	164	91
B1/B3	Hexanoyl sphingosine	Carbenoxelone	122	164	78
B1/B3	Lyral	Carbenoxelone	120	164	82
B1/B3	Castor oil MEA	Carbenoxelone	110	164	78
B1/B3	Hexanoyl sphingosine	Amsacrine	122	134	92
B1/B3	Hexanoyl sphingosine	Eliadic acid	122	144	85
B1/B3	Alpha ionone	Amsacrine	101	134	78
B1/B3	1,2 dioctanoyl-sn-glycero-3-phosphoethanolamide	Glyccyrrhetinic acid	95	92	69
B1/B4	Naringenin	2- hydroxy steric acid	95	112	78
B1/B4	Hexanoyl sphingosine	2- hydroxy steric acid	99.3	112	77
B1/B4	Lyral	Hexadecanoic acid	120	95	69
B1/B4	Castor oil MEA	Hexadecanedioic acid	110	125	82
B1/B4	Hexanoyl sphingosine	Isostearic acid	122	146	93
B1/B4	Oleoyl betaine	Hexadecanedioic acid	99.5	125	80
B1/B5	Hexanoyl sphingosine	Cocoyl hydorxyethylimidazolin e	99	102	68
B1/B5	Farnesol	Ketokonazole	98	111	84
B1/B5	Hexanoyl sphingosine	Miconazole	99	101	56
B1/B5	Hexanoyl sphingosine	Ketoconazole	99	99	65
B1/B5	Hexanoyl sphingosine	Lauryl hydroxyethylimiazoline	99	98	51
B1/B5	Utrecht-2	Amino benzotriazole	122	105	83
B1/B5	Hexanoyl sphingosine	3,4-dihydro-2 quinolinone	122	102	89
B1/B5	Hexanoyl sphingosine	Amino benzotriazole	122	126	85
B1/B5	Castor oil MEA	Lauryl hydroxyethylimiazoline	110	98	56
B1/B5	Hexanoyl sphingosine	Climbazole	122	98	83
B1/B5	Hexanoyl sphingosine	Miconazole	122	99	78
B1/B5	Hexanoyl sphingosine	Ketoconazole	122	110	90

B1/B5	Oleoyl beatine	ketoconazole	96	116	81
B1/B5	Utrecht-2	Lauryl hydroxyethylimiazoline	122	98	57
B1/B5	Alpha-demascone	Oleoyl hydroxyethylimiazoline	112	73	76
B1/B5	Alpha-ionone	Lauryl hydroxyethylimiazoline	101	98	49
B1/B5	Alpha-ionone	Oleoyl hydroxyethylimiazoline	101	73	75
B2/B3	Phosphatidyl choline	Glycyrrhetinic acid	98	92	73
B2/B4	Phosphatidyl choline	2-hydroxy steric acid	98	82	70
B2/B5	Phosphatidyl choline	Climbazole	98	102	82
B2/B5	Phosphatidyl choline	Miconazole	98	111	92
B2/B5	Phosphatidyl choline	Ketoconazole	98	101	89
B2/B5	Phosphatidyl choline	Lauryl hydorxyimidazoline	98	106	82
B3/B4	Amscarine	2-hydroxy steric acid	102	82	75
B3/B4	Myristic acid	2-hydroxy steric acid	110	82	78
B3/B5	Amscarine	Aminobenzotriazole	102	98	84
B3/B5	Amscarine	Dimethyl imidazoline	102	112	94
B3/B5	Myristic acid	Climbazole	110	102	82
B4/B5	Linseed oil	Lauryl hydroxyethyl imidazoline	98	73	57
B4/B5	2-hydroxystearic acid	Ketaconazole	92	109	77
B4/B5	Linseed oil	Oleoyl hydorxyethylimdazoline	98	92	75
B4/B5	2-hydroxystearic acid	Coumarin	92	96	70

Synergy of Tgase inhibition with tertiary combinations of boosters

To investigate synergistic inhibition of Tgase expression by combinations of 3 different classes of boosters with retinol, selected combinations of compounds were tested. The concentrations tested were one log order of magnitude less than the concentration required for minimal inhibition of Tgase activity (i.e. IC_{20}). The compounds were tested alone and in combination and the % inhibition of Tgase is given for each compound and the combination. The following examples give the synergistic combinations in all possible tertiary combinations (B1/B2/B3;B1/B2/B4;B1/B2/B5;

B1/B3/B4;B1/B3/B5; B1/B4/B5; B2/B3/B4; B2/B3/B5; B2/B4/B5;B3/B4/B5). The % inhibition of the combination is more than the inhibition of each compound added together, which indicates synergy (i.e. Inhibition by combination is greater than inhibition by compound 1 + compound 2 + compound 3). All the examples of teritiary combinations of boosters given in the following table synergistically inhibited Tgase in the presence of 10-7M retinol.

Compound 1	Compound 2	Compound 3	TG as % C Compd 1	TG as % C Compd 2	TG as % C Compd 3	TG as % C Combo
B1/B2/B3 combinations:						
Phosphatidyl Choline	Glycyrrhetinic Acid	Castor oil Methyl Ester Acid (MEA)	88	91	85	53
Phosphatidyl Choline	Glycyrrhetinic Acid	Echinacea	88	91	119	52
Phosphatidyl Choline	Glycyrrhetinic Acid	Naringenin	88	91	94	52
Phosphatidyl Choline	Glycyrrhetinic Acid	Acetyl sphingosine (C2 Ceramide)	88	91	99	58
Phosphatidyl Choline	Glycyrrhetinic Acid	Farnesol	88	91	118	49
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	a-Damascone	81	91	89	58
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Phosphatidyl Choline	Naringenin	81	88	94	66
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Amsacrine-HCl	Linoleoyl Monoethanolamide (LAMEA)	81	79	127	60
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Amsacrine-HCl	Palmitamide Monoethanolamide	81	79	95	63
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	a-Damascone	81	91	89	58
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Naringenin	81	91	94	75
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Echinacea	81	91	119	77
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Dimethyl imidazolinone	81	91	87	67
Castor oil Methyl Ester Acid (MEA)	Carbenoxelone	Phosphatidyl Choline	85	95	88	63

B1/B2/B4 Combinations:

B1/B2/B5 Combinations:

Phosphatidyl Choline	Climbazole	Echinacea	88	84	119	75
Phosphatidyl Choline	Climbazole	Naringenin	88	84	94	83
Phosphatidyl Choline	Climbazole	Geraniol	88	84	105	76
Phosphatidyl Choline	Climbazole	Farnesol	88	84	118	82
Phosphatidyl Choline	Climbazole	Acetyl sphingosine (C2 Ceramide)	88	84	99	82
Phosphatidyl Choline	Miconazole	a-Ionone	88	92	88	70
Phosphatidyl Choline	Miconazole	Castor oil Methyl Ester Acid (MEA)	88	92	85	72

B1/B3/B4 Combinations:

Amsacrine-HCl	Dimethyl imidazolinone	Elaidic acid	79	87	93	0
□-Ionone	Amsacrine-HCl	12-Hydroxystearic acid	68	79	95	62
Lyrial	Hexadecanedioic acid	Vanillin	97	90	134	81
Hexanoyl sphingosine (C6 Ceramide)	Isostearic acid	Glycyrrhetinic Acid	104	87	91	58

B1/B3/B5 Combinations:

Amsacrine-HCl	Dimethyl imidazolinone	2-Hydroxyquinoline(Carbostyril)	79	87	95	32
Amsacrine-HCl	Dimethyl imidazolinone	Lauryl hydroxyethylimidazoline	79	87	52	-13
Amsacrine-HCl	Dimethyl imidazolinone	Quercetin	79	87	92	-24
Amsacrine-HCl	Dimethyl imidazolinone	Oleoyl hydroxyethlimidazoline	79	87	76	39
Amsacrine-HCl	Dimethyl imidazolinone	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	79	87	94	32
Amsacrine-HCl	Dimethyl imidazolinone	Coumarin	79	87	80	30
Hexanoyl sphingosine (C6 Ceramide)	Carbenoxolone	Oleoyl hydroxyethlimidazoline	104	88	76	64
Hexanoyl sphingosine (C6 Ceramide)	3,4,-Dihydro-2(1H)-quinolinone(Hydrocarbostyril)	Vanillin	104	90	134	62
Amsacrine-HCl	Amino Benzotriazole	Echinacea	79	105	119	48
Hexanoyl sphingosine (C6 Ceramide)	Amino Benzotriazole	Sphingomyelin	104	105	60	69
Amsacrine-HCl	Amino Benzotriazole	Acetyl sphingosine (C2 Ceramide)	79	105	99	-7
□-Ionone	Amsacrine-HCl	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	68	79	94	54

Utrecht-2	Carbenoxolone	Quercetin	76	88	92	74
Utrecht-2	Carbenoxolone	Oleoyl [ ] hydroxyethlimidazoline	76	88	76	69
Utrecht-2	Carbenoxolone	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	76	88	94	73
Utrecht-2	Carbenoxolone	3,4,-Dihydro-2(1H)-quinolinone(Hydrocarbostyril)	76	88	90	70
Myristic Acid	Climbazole	Geraniol	79	84	105	74
Myristic Acid	Climbazole	□-Damascone	79	84	89	73
Myristic Acid	Climbazole	Acetyl sphingosine (C2 Ceramide)	79	84	99	70
Oleyl Betaine	Ketoconazole	Carbenoxolone	62	85	88	78
Oleyl Betaine	Ketoconazole	Glycyrrhetinic Acid	62	85	91	71
Oleyl Betaine	Ketoconazole	Linoleic Acid	62	85	11	83
Oleyl Betaine	Ketoconazole	Linolenic Acid	62	85	208	80
Hexanoyl sphingosine (C6 Ceramide)	3,4,-Dihydro-2(1H)-quinolinone(Hydrocarbostyril)	Vanillin	104	90	134	62

B1/B4/B5 Combinations:

Elaidic acid	2-Hydroxyquinoline (Carbostyril)	Castor oil Methyl Ester Acid (MEA)	93	95	85	75
Elaidic acid	2-Hydroxyquinoline (Carbostyril)	Naringenin	93	95	94	86
Elaidic acid	2-Hydroxyquinoline (Carbostyril)	a-Damascone	93	95	89	80
Elaidic acid	2-Hydroxyquinoline (Carbostyril)	Farnesol	93	95	118	82
Elaidic acid	2-Hydroxyquinoline (Carbostyril)	Crocetin	93	95	90	78

B2/B3/B4 Combinations:

1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	12-Hydroxystearic acid	81	91	95	57
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Linseed oil	81	91	103	62
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Elaidic acid	81	91	93	75
Phosphatidyl Choline	2-Hydroxystearic acid	Arachidonic Acid (Na+ salt)	88	83	78	60

B2/B3/B5 Combinations:

Phosphatidyl Choline	Climbazole	Linolenic Acid	88	84	208	84
Phosphatidyl Choline	Climbazole	Arachidonic Acid (Na+ salt)	88	84	78	83
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Amsacrine-HCl	Climbazole	81	79	84	58
1,2-dioctanoyl-sn-	Amsacrine-HCl	7H-	81	79	94	59

glycero-3-phosphoethanolamide		Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one				
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	3,4,-Dihydro-2(1H)-quinolinone(Hydrocarbostyril)	81	91	90	56
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	2-Hydroxyquinoline(Carbostyril)	81	91	95	75
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Amino Benzotriazole	81	91	105	72
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazoline	81	91	52	79
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Quercetin	81	91	92	73
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Climbazole	81	91	84	54
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Clotrimazole	81	91	79	42
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Miconazole	81	91	82	43
B2/B4/B5 Combinations:						
Phosphatidyl Choline	2-Hydroxystearic acid	Amino Benzotriazole	88	83	105	77
Phosphatidyl Choline	2-Hydroxystearic acid	Lauryl hydroxyethylimidazoline	88	83	52	74
Phosphatidyl Choline	2-Hydroxystearic acid	Quercetin	88	83	92	69
Phosphatidyl Choline	2-Hydroxystearic acid	Oleoyl hydroxyethlimidazoline	88	83	76	75
Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	88	83	94	79
Phosphatidyl Choline	Climbazole	Elaidic acid	88	84	93	81
B3/B4/B5 Combinations:						
Elaidic acid	2-Hydroxyquinoline (Carbostyril)	Carbenoxolone	93	95	88	69
Elaidic acid	2-Hydroxyquinoline (Carbostyril)	Vanillin	93	95	134	81
Amsacrine-HCl	Amino Benzotriazole	Linseed oil	79	105	103	45
Myristic Acid	Climbazole	12-Hydroxystearic acid	79	84	95	81
Myristic Acid	Climbazole	Linseed oil	79	84	103	81
Elaidic acid	2-Hydroxyquinoline (Carbostyril)	Arachidonic Acid (Na+ salt)	93	95	78	63

Synergy of Tgase inhibition with quaternary combinations of boosters

To investigate synergistic inhibition of Tgase expression by combinations of 4 different classes of boosters with retinol, selected combinations of compounds were tested. The concentrations tested were one log order of magnitude less than the concentration required for minimal inhibition of Tgase activity (i.e. IC_{20}).

The compounds were tested alone and in combination and the % inhibition of Tgase is given for each compound and the combination. The following examples give the synergistic combinations in all possible quaternary combinations (B1/B2/B3/B4; B1/B2/B3/B5; B1/B2/B4/B5; B1/B3/B4/B5; B2/B3/B4/B5;). Synergy was confirmed if the difference in % inhibition of the combination (of 4 boosters) is more than 30% that of the inhibition by 3 booster combinations (i.e. % inhibition of 4 booster combo is equal to or greater than % inhibition of 3 booster combo + 30%). All the quaternary combinations of boosters shown in the table given below showed synergy.

Compound 1	Compound 2	Compound 3	Compound 4	Quarter-nary TG (%C)	Tertiary (1-3 combo; TG %C)	Differ-ence (<30%=synergy)
B1/B2/B3/B4 Combination:						
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	12-Hydroxy-stearic acid	21	64	42
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	12-Hydroxy-stearic acid	15	57	41
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	12-Hydroxy-stearic acid	-3	40	43
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Isostearic acid	5	40	35
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-	Amsacrine-HCl	12-Hydroxy-stearic acid	-3	42	45

	amide					
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Elaidic acid	8	42	34
Hexanoyl sphingosine (C6 Ceramide)	TCC	Glycyrrhetinic Acid	Isostearic acid	7	54	47
Lyrial	TCC	Vanillin	Hexadecan-edioic acid	10	48	38
Cocoyl hydroxyethylimid-azoline	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Isostearic acid	0	37	37
Cocoyl hydroxyethylimid-azoline	Phosphatidyl Choline	Arachidonic Acid (Na+ salt)	2-Hydroxy-stearic acid	-1	37	38
Cocoyl hydroxyethylimid-azoline	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Linseed oil	-2	45	47

B1/B2/B3/B5 Combination:

Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Climbazole	20	64	44
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Clotrimazole	26	64	38
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole	9	64	55
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Ketoconazole	5	64	59
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazoline	15	64	49
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	2	64	61
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Glycyrrhetinic Acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	25	64	39
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	12-Hydroxystearic acid	18	62	44
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Climbazole	22	62	40
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Clotrimazole	24	62	38
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole	13	62	50
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Ketoconazole	12	62	50
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazoline	14	62	49
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	3	62	59
Echinacea	Phosphatidyl Choline	Glycyrrhetinic Acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	24	62	39
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole	1	57	56
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Ketoconazole	22	57	34
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazoline	10	57	46
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	2	57	54
Naringenin	Phosphatidyl Choline	Glycyrrhetinic Acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-	15	57	42

			7-one			
Palmitamide Monoethanolamide	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole	-2	39	41
Palmitamide Monoethanolamide	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	6	39	33
Farnesol	Phosphatidyl Choline	Glycyrrhetinic Acid	Miconazole	3	43	40
Farnesol	Phosphatidyl Choline	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	6	43	37
Geraniol	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Miconazole	11	47	36
Geraniol	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Oleoyl hydroxyethlimidazoline	3	47	44
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Climbazole	2	40	37
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Miconazole	5	40	35
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Ketoconazole	0	40	40
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Lauryl hydroxyethylimidazoline	-2	40	41
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Oleoyl hydroxyethlimidazoline	5	40	35
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	1	40	39
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Climbazole	7	42	35
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Clotrimazole	10	42	32
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Miconazole	5	42	37
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Ketoconazole	11	42	32
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Lauryl hydroxyethylimidazoline	-4	42	46
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Oleoyl hydroxyethlimidazoline	5	42	37
Linoleoyl Monoethanolamide (LAMEA)	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-	Amsacrine-HCl	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	8	42	35

	amide					
Palmitamide Monoethanolamide	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Miconazole	13	43	30
Palmitamide Monoethanolamide	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Oleoyl hydroxyethlimidazoline	3	43	40
Alpha-Damascone	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Miconazole	11	48	37
Alpha-Damascone	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Ketoconazole	13	48	34
Alpha-Damascone	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Lauryl hydroxyethylimidazoline	15	48	33
Alpha-Damascone	1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Amsacrine-HCl	Oleoyl hydroxyethlimid-azoline	3	48	45
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	12-Hydroxystearic acid	3	55	52
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	Climbazole	6	55	49
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	Miconazole	-2	55	57
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	Ketoconazole	1	55	54
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	Lauryl hydroxyethylimi-dazoline	4	55	51
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	Oleoyl hydroxyethlimidazoline	3	55	52
Castor oil Methyl Ester Acid (MEA)	Phosphatidyl Choline	Carbenoxolone	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	11	55	44
Naringenin	Phosphatidyl Choline	Linoleic Acid	Climbazole	-1	45	46
Geraniol	Phosphatidyl Choline	Linoleic Acid	Climbazole	1	40	39
Acetyl sphingosine (C2 Ceramide)	Phosphatidyl Choline	Linoleic Acid	Climbazole	0	40	40
Acetyl sphingosine (C2 Ceramide)	Phosphatidyl Choline	Linolenic Acid	Climbazole	10	40	30
Dimethyl imidazolinone	TCC	Amsacrine-HCl	Elaidic acid	14	47	33
Dimethyl imidazolinone	TCC	Amsacrine-HCl	Quercetin	12	44	32
Dimethyl imidazolinone	TCC	Amsacrine-HCl	Coumarin	14	58	44
Hexanoyl sphingosine (C6 Ceramide)	TCC	Glycyrrhetinic Acid	Amino Benzotriazole	8	48	40
Alpha-Damascone	TCC	Myristic Acid	Climbazole	10	44	34

B1/B2/B4/B5 Combination:						
Lyrial	Vanilin	Hexadecanedioic acid	Miconazole	12	48	36
Lyrial	Vanilin	Hexadecanedioic acid	Oleoyl hydroxyethlimidazoline	4	48	45
Crocetin	TCC	Elaidic acid	2-Hydroxyquinoline(Carbostyril)	11	48	37
Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	12-Hydroxystearic acid	Amino Benzotriazole	14	48	33
Dimethyl imidazolinone	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	2	44	42
Melinamide	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	5	44	39
Geranyl geraniol	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	9	44	35
Cocoyl hydroxyethylimidazoline	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	-8	44	52
Acetyl sphingosine (C2 Ceramide)	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	10	44	34
Crocetin	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	10	44	34
N,N-Diethyl Cocamide(Cocamide DEA)	Phosphatidyl Choline	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	4	44	40
Cocoyl hydroxyethylimidazoline	Phosphatidyl Choline	Elaidic acid	Climbazole	-4	30	34
B1/B3/B4/B5 Combination:						
Dimethyl imidazolinone	Amsacrine-HCl	Elaidic acid	Miconazole	7	47	40
Dimethyl imidazolinone	Amsacrine-HCl	Elaidic acid	Ketoconazole	6	47	41
Dimethyl imidazolinone	Amsacrine-HCl	Elaidic acid	Oleoyl hydroxyethlimidazoline	3	47	44
Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Clotrimazole	20	54	34
Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Miconazole	10	54	43
Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Lauryl hydroxyethylimidazoline	20	54	33
Hexanoyl sphingosine (C6 Ceramide)	Glycyrrhetinic Acid	Isostearic acid	Oleoyl hydroxyethlimidazoline	5	54	48
Crocetin	Linoleic Acid	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	0	48	48
Crocetin	Linolenic Acid	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	-2	48	50
Castor oil Methyl Ester Acid (MEA)	Linoleic Acid	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	-1	31	32
Cocoyl hydroxyethylimid-azoline	Carbenoxolone	Elaidic acid	2-Hydroxyquinoline (Carbostyril)	-6	28	34

B2/B3/B4/B5 Combination:						
1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Isostearic acid	Ketoconazole	4	37	33
1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Isostearic acid	Oleoyl hydroxyethlimidazoline	6	37	31
Phosphatidyl Choline	Arachidonic Acid (Na+ salt)	2-Hydroxystearic acid	Miconazole	6	37	31
Phosphatidyl Choline	Arachidonic Acid (Na+ salt)	2-Hydroxystearic acid	Oleoyl hydroxyethlimidazoline	5	37	32
1,2-dioctanoyl-sn-glycero-3-phosphoethanolamide	Glycyrrhetinic Acid	Linseed oil	Miconazole	-1	45	47
1,2-dioctanoyl-sn-glycero-3-phosphoethanol-amide	Glycyrrhetinic Acid	Linseed oil	Oleoyl hydroxyethlimidazoline	7	45	38
Phosphatidyl Choline	Carbenoxolone	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	8	44	36
Phosphatidyl Choline	Linoleic Acid	2-Hydroxystearic acid	7H-Benzimidazo[2,1-a]Benz[de]-isoquinolin-7-one	-3	44	47
Phosphatidyl Choline	Glycyrrhetinic Acid	Elaidic acid	Climbazole	-3	30	33
Phosphatidyl Choline	Linoleic Acid	Elaidic acid	Climbazole	-2	30	32

Cosmetically Acceptable Vehicle

The composition according to the invention also comprises a cosmetically acceptable vehicle to act as a dilutant, dispersant or carrier for the active components in the composition, so as to facilitate their distribution when the composition is applied to the skin.

Vehicles other than or in addition to water can include liquid or solid emollients, solvents, humectants, thickeners and powders. An especially preferred non-aqueous carrier is a polydimethyl siloxane and/or a polydimethyl phenyl siloxane. Silicones of this invention may be those with viscosities ranging anywhere from about 10 to 10,000,000 centistokes at 25°C. Especially desirable are mixtures of low

and high viscosity silicones. These silicones are available from the General Electric Company under trademarks Vicasil, SE and SF and from the Dow Corning Company under the 200 and 550 Series. Amounts of silicone which can be utilised in the compositions of this invention range anywhere from 5 to 95%, preferably from 25 to 90% by weight of the composition.

Optional Skin Benefit Materials and Cosmetic Adjuncts

An oil or oily material may be present, together with an emulsifier to provide either a water-in-oil emulsion or an oil-in-water emulsion, depending largely on the average hydrophilic-lipophilic balance (HLB) of the emulsifier employed.

Various types of active ingredients may be present in cosmetic compositions of the present invention. Various types of active ingredients may be present in cosmetic compositions of the present invention. Actives are defined as skin or hair benefit agents other than emollients and other than ingredients that merely improve the physical characteristics of the composition. Although not limited to this category, general examples include sunscreens, skin lightening agents, and tanning agents.

Sunscreens include those materials commonly employed to block ultraviolet light. Illustrative compounds are the derivatives of PABA, cinnamate and salicylate. For example, octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone (also known as oxybenzone) can be used. Octyl

methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available under the trademarks, Parsol MCX and Benzophenone-3, respectively.

The exact amount of sunscreen employed in the emulsions can vary depending upon the degree of protection desired from the sun's UV radiation.

Another preferred optional ingredient is selected from essential fatty acids (EFAs), i.e., those fatty acids which are essential for the plasma membrane formation of all cells, in keratinocytes EFA deficiency makes cells hyperproliferative. Supplementation of EFA corrects this. EFA's also enhance lipid biosynthesis of epidermis and provide lipids for the barrier formation of the epidermis. The essential fatty acids are preferably chosen from linoleic acid, γ-linolenic acid, homo- γ-linolenic acid, columbinic acid, eicosa-(n-6,9,13)-trienoic acid, arachidonic acid, γ-linolenic acid, timnodonic acid, hexaenoic acid and mixtures thereof.

Emollients are often incorporated into cosmetic compositions of the present invention. Levels of such emollients may range from about 0.5% to about 50%, preferably between about 5% and 30% by weight of the total composition. Emollients may be classified under such general chemical categories as esters, fatty acids and alcohols, polyols and hydrocarbons.

Esters may be mono- or di-esters. Acceptable examples of fatty di-esters include dibutyl adipate, diethyl sebacate,

diisopropyl dimerate, and dioctyl succinate. Acceptable branched chain fatty esters include 2-ethyl-hexyl myristate, isopropyl stearate and isostearyl palmitate. Acceptable tribasic acid esters include triisopropyl trilinoleate and trilauryl citrate. Acceptable straight chain fatty esters include lauryl palmitate, myristyl lactate, oleyl eurcate and stearyl oleate. Preferred esters include coco-caprylate/caprate (a blend of coco-caprylate and coco-caprate), propylene glycol myristyl ether acetate, diisopropyl adipate and cetyl octanoate.

Suitable fatty alcohols and acids include those compounds having from 10 to 20 carbon atoms. Especially preferred are such compounds such as cetyl, myristyl, palmitic and stearyl alcohols and acids.

Among the polyols which may serve as emollients are linear and branched chain alkyl polyhydroxyl compounds. For example, propylene glycol, sorbitol and glycerin are preferred. Also useful may be polymeric polyols such as polypropylene glycol and polyethylene glycol. Butylene and propylene glycol are also especially preferred as penetration enhancers.

Exemplary hydrocarbons which may serve as emollients are those having hydrocarbon chains anywhere from 12 to 30 carbon atoms. Specific examples include mineral oil, petroleum jelly, squalene and isoparaffins.

Another category of functional ingredients within the cosmetic compositions of the present invention are thickeners. A thickener will usually be present in amounts anywhere from 0.1 to 20% by weight, preferably from about 0.5% to 10% by weight of the composition. Exemplary thickeners are cross-linked polyacrylate materials available under the trademark Carbopol from the B.F. Goodrich Company. Gums may be employed such as xanthan, carrageenan, gelatin, karaya, pectin and locust beans gum. Under certain circumstances the thickening function may be accomplished by a material also serving as a silicone or emollient. For instance, silicone gums in excess of 10 centistokes and esters such as glycerol stearate have dual functionality.

Powders may be incorporated into the cosmetic composition of the invention. These powders include chalk, talc, Fullers earth, kaolin, starch, smectite clays, chemically modified magnesium aluminum silicate, organically modified montmorillonite clay, hydrated aluminum silicate, fumed silica, aluminum starch octenyl succinate and mixtures thereof.

Other adjunct minor components may also be incorporated into the cosmetic compositions. These ingredients may include coloring agents, opacifiers and perfumes. Amounts of these materials may range anywhere from 0.001% up to 20% by weight of the composition.

Use of the Composition

The composition according to the invention is intended primarily as a product for topical application to human skin, especially as an agent for conditioning and smoothening the skin, and preventing or reducing the appearance of wrinkled or aged skin.

In use, a small quantity of the composition, for example from 1 to 5ml, is applied to exposed areas of the skin, from a suitable container or applicator and, if necessary, it is then spread over and/or rubbed into the skin using the hand or fingers or a suitable device.

Product Form and Packaging

The topical skin treatment composition of the invention can be formulated as a lotion, a fluid cream, a cream or a gel. The composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or fluid cream can be packaged in a bottle or a roll-ball applicator, or a capsule, or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar.

The invention accordingly also provides a closed container containing a cosmetically acceptable composition as herein defined.

CLAIMS

1. A skin care composition comprising:
   a. from 0.001% to 10% of a retinoid;
   b. a combination of at least 2 retinoid boosters belonging to classes B1 to B5 in an amount of from 0.0001% to 50% where the ratios of the two boosters to each other in the range of is 1:1000 to 1000:1;
   c. a cosmetically acceptable vehicle.

2. The skin care composition of claim 1 where the combination of boosters comprises at least three boosters belonging to the classes B1 to B5 in an amount of from 0.0001% to 50%.

3. The skin care composition of claim 1 or claim 2 where the second composition has a combination of at least 4 boosters belonging to the classes B1 to B5 in an amount of from 0.0001% to 50%.

4. The skin care composition of any of the preceding claims where the second composition has a combination of all the 5 classes of boosters belonging to the classes B1 to B5.

5. A cosmetic method of conditioning skin, the method comprising applying topically to the skin the product of any one of claims 1 through to 5.

6. A cosmetic method of mimicking the effect on skin or retinoic acid, the method comprising applying to the skin the product of any one of claims 1-5.

7. A skin care composition comprising:

a. a combination of at least 2 retinoid boosters belonging to classes B1 to B5 in an amount of from 0.0001% to 50% where the ratios of the two boosters to each other in the range of is 1:1000 to 1000:1;

b. a cosmetically acceptable vehicle.

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(54) Title: SKIN CONDITIONING COMPOSITIONS CONTAINING COMPOUNDS FOR MIMICKING THE EFFECT ON SKIN OF RETINOIC ACID

(57) Abstract: A skin care product comprising from about 0.001 % to about 10 % of a retinoid, in combination with 0.0001 % to about 50 % of a combination of retinoid boosters.

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IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 803 248 A (UNILEVER PLC ;UNILEVER NV (NL)) 29 October 1997 (1997-10-29) claims 1-9; examples 5-10 & US 5 716 627 A 10 February 1998 (1998-02-10) cited in the application	1-7
X	EP 0 803 247 A (UNILEVER PLC ;UNILEVER NV (NL)) 29 October 1997 (1997-10-29) example 9 & US 5 693 330 A 2 December 1997 (1997-12-02) cited in the application	1-7
X	WO 98 13020 A (UNILEVER PLC ;UNILEVER NV (NL)) 2 April 1998 (1998-04-02) claims 1-10; examples 14B,15D	1-7

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 December 2002	16/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fischer, J.P.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/07234

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 759 556 A (RAWLINGS ANTHONY VINCENT ET AL) 2 June 1998 (1998-06-02) cited in the application example 10	1-7
X,P	WO 01 08650 A (UNILEVER PLC ;LEVER HINDUSTAN LTD (IN); UNILEVER NV (NL)) 8 February 2001 (2001-02-08) the whole document	1-7
X	US 5 665 367 A (BURGER ALLAN ROBERT ET AL) 9 September 1997 (1997-09-09) cited in the application claims 1-6; example 10	1-7
X	US 5 811 110 A (GRANGER STEWART PATON ET AL) 22 September 1998 (1998-09-22) cited in the application claims 1-9; example 14	1-7
X	US 5 599 548 A (GRANGER STEWART P ET AL) 4 February 1997 (1997-02-04) cited in the application claims 1-12; example 14	1-7
X	US 5 756 109 A (RAWLINGS ANTHONY VINCENT ET AL) 26 May 1998 (1998-05-26) cited in the application example 9	1-7
X	WO 93 09805 A (INOCOSM LAB) 27 May 1993 (1993-05-27) claims 1,5; example 1	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/07234

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: –
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-7 relate to an extremely large number of possible compositions. In fact, the claims contain so many possible combinations of structurally unrelated compounds that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for the general idea underlying the application only.

Claims searched completely : none

Claims searched uncompletely: 1-7

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 01/07234

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